Development and Application of MRI Techniques for Non-Invasive Assessment of Blood-Cerebrospinal Fluid Barrier Function

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Research Degree: Division of Medicine

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08/10/2022
Abstract

The choroid plexus (CP) tissue forms the blood-cerebrospinal fluid barrier (BCSFB) - a unique interface which plays a critical role in effective homeostasis of the central nervous system. To date, exploration of the BCSFB’s role in health and disease has been hindered by a lack of non-invasive, translatable methodologies. The recent development of BCSFB-ASL MRI by Evans et al. has permitted the non-invasive, surrogate measurement of BCSFB function. The work presented herein develops and applies the BCSFB-ASL method to investigate BCSFB function in rodent models of ageing and disease.

Chapter 2 describes a novel platform for simultaneous recording of BCSFB function and brain tissue perfusion using interleaved echo-time ASL, which provided insight into alterations of vessel tone at the BBB and BCSFB under the influence of pharmacological agents, as well as how reactivity towards a vasopressin challenge is impaired in the aged mouse brain.

In Chapter 3, I reproduce, optimise, and characterise the BCSFB-ASL MRI approach on a Bruker 9.4T system, that was heretofore applied only on an Agilent 9.4T MRI system. This work seeks to utilise the improved hardware and software on the Bruker system to increase measurement precision with minimised scan times.

Chapter 4 describes efforts to further characterise the contributing sources and kinetics of ultra-long echo-time ASL signals arising from brain-wide CSF regions. These experiments seek to determine the reliability of the estimated labelled blood water delivery rates, alongside potential factors which may contribute to the appearance of these signals, in regions distal to the caudal lateral ventricles.

In Chapter 5, BCSFB function was then investigated in the context of systemic hypertension. Spontaneously hypertensive rats displayed a reduction in BCSFB function, which highlights the potential for such measures to serve as a sensitive early biomarker for hypertension-driven neurodegeneration.

Overall, we demonstrate the scope of BCSFB-ASL to capture changes to BCSFB function, which not only has value in providing a useful biomarker for downstream neurodegeneration, but
also provides an insight into mechanisms which may increase the brain’s susceptibility towards neurodegenerative outcomes.
Impact Statement

With global increases in population ageing, there has been an increased prevalence of neurodegenerative disease. Figures from the World Health Organisation indicate that, with 55 million people suffering worldwide, dementia is now one of the biggest healthcare burdens. Despite an improvement in our knowledge of the risk factors contributing to neurodegeneration, there is still an inadequate understanding in the underlying mechanisms leading to disease, as well as limited biomarkers that can sensitively detect early signs of neurodegenerative pathology.

The blood-cerebrospinal fluid barrier (BCSFB) is a critical, yet understudied, interface in the brain, which has been shown to display a selective vulnerability to stressors associated with neurodegenerative disease. The work presented in this thesis seeks to develop and apply the recently developed BCSFB-ASL MRI approach to assess how BCSFB function is altered between healthy and altered brain states, such as the aged brain or during systemic hypertension, in a non-invasive manner.

This body of work will benefit both national and international academic centres that are working towards better understanding pathophysiological mechanisms underlying early changes that occur during neurodegeneration. Most importantly, the data presented in this thesis highlights the value of BCSFB-ASL, and adaptations of this methodology, to provide a novel and non-invasive method that enables the study of the CP-BCSFB system in a readily translatable manner. As such, this in-vivo tool may aid the early detection of AD, by assessing early signs of functional impairment of the BCSFB.

Furthermore, the dissemination of work presented in this thesis by means of published research articles, presentations at national and international scientific conferences, and outreach work, will inevitably drive more interest in the field of MR biomarkers of neuroimaging, as well as the study of the choroid plexus, BCSFB, and cerebrospinal fluid – a field undergoing a resurgence due to the major implications for brain health and waste clearance. Of note, we were invited to submit work to Frontiers in Molecular Neuroscience, which was to be included in their Special Research Topic: ‘Cerebrospinal Fluid Dynamics and
Intracranial Pressure Elevation – Novel Insights on Molecular and Physiological Mechanisms, and Implications for Neurological Disease’.

Presenting my work at an international conference has also facilitated a collaboration with the Champalimaud Foundation (Lisbon, Portugal). This collaborative project seeks to capture changes in BCSFB function, and other markers of cerebrovascular health and cognitive function, across multiple timepoints across the lifespan of a rodent model of AD. By studying this biomarker across multiple disease timepoints in the context of AD, we are able to make further progress towards the goal of early disease diagnosis in patients.

Publications


Selected conference oral presentations

- Cold Spring Harbor Laboratory (2021) – Virtual Brain Barriers meeting.
- ISMRM Annual Meeting (2021) – Hybrid meeting, presented virtually.
- European Molecular Imaging Meeting (Thessaloniki, Greece, 2022) – European Society for Molecular Imaging.
Selected conference poster presentations

- Postgraduate Symposium (2021) – British & Irish Chapter of ISMRM (special attendance from Iberian chapter).
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Acknowledgements

Being taken on as a PhD candidate by my supervisor, Dr. Jack A. Wells, has been a life-changing experience. Whilst pushing me to be the best version of myself, his kind-hearted and supportive nature has kept me motivated and inspired throughout my PhD, and through some of my toughest times. I would also like to thank Prof. Mark F. Lythgoe for always challenging my research, as well as shaping the way I communicate to effectively rally a crowd, which together, has enabled me to become a more confident public speaker. In addition, Dr. David L. Thomas has provided invaluable insights into my work, particularly with troubleshooting. I am grateful to my loving wife Rianne, alongside my family, friends, and my team at UCL’s Centre of Advanced Biomedical Imaging for being pillars of support; their love and friendship has kept me level-headed throughout this process. Last, but by no means least, I extend my gratitude to the Alzheimer’s Society for their generous funding throughout the duration of my PhD.

This thesis is dedicated to my grandma. I wish you could have been here to see me finish this journey. Rest in peace.
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1. Chapter 1 – Literature Review

a. Introduction

Together, the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) work synchronously to maintain the homeostatic balance of the central nervous system (CNS) for optimal function. Historically, a plethora of studies have utilised non-invasive methods such as fMRI-BOLD and arterial spin labelling (ASL) as a biomarker for cerebrovascular health, thereby providing a surrogate measure of blood-brain barrier (BBB) health and integrity. On the other hand, our understanding of the BCSFB in physiology and disease has been limited by the absence of a non-invasive measurement technique to probe the functionality of this locus. Directly addressing this need, the recent development of the blood-cerebrospinal fluid barrier arterial spin labelling (BCSFB-ASL) technique by Evans et al. (2020) provides a non-invasive, surrogate measurement of BSCFB function by using non-contrast-enhanced MRI, and an ultra-long 220 ms echo time parameter to quantify rates of water delivery from arterial blood to ventricular cerebrospinal fluid (CSF)\(^1\) (introductory schematic: Figure 1). The work presented within this thesis will aim to apply and optimise the BCSFB-ASL methodology in order to non-invasively assess BCSFB function in the healthy brain, as well as investigating how BCSFB function is deranged in altered brain states, e.g. ageing and hypertension. Furthermore, by further developing the BCSBF-ASL methodology, we aim to broaden the scope of applications of the methodology, e.g. for the study of ultra-long TE ASL signals in other brain regions rich in CSF.

In this Chapter, I will provide a literature review of the BBB and BCSFB, as well as the measurement of both parenchymal cerebral blood flow (CBF) using traditional-ASL and rates of BCSFB-mediated water delivery using BCSFB-ASL, which form the backbone to the studies performed within this body of work.
Figure 1 – BCSFB-ASL introductory schematic: this technique works by labelling arterial blood water in the brain’s feeding arteries and tuning the MRI acquisition (using TE = 220 ms) to measure signal isolated from labelled blood water that has been transported to the CSF via the BCSFB (blue arrow), thereby providing a surrogate measure of BCSFB function. Schematic taken from Evans et al., 2020, Figure 1a. Note: the feeding arteries displayed here are for visual representation of the “labelling” used in arterial spin labelling MRI. BCSFB-ASL methods implemented within this thesis specifically utilise the FAIR-ASL labelling scheme with a broad labelling pulse covering the entire brain, as described in Figure 3.

b. Blood-Brain Barrier (BBB)

The blood-brain barrier (BBB) and blood-cerebrospinal-fluid barrier (BCSFB) are structurally and functionally distinct networks of cells within the brain (Figure 2). Together, these transport interfaces for blood-central nervous system (CNS) exchange contribute to the homeostatic regulation of an optimal environment for neural signalling. At the BBB, endothelial cells of the capillary wall form a semipermeable barrier, and are associated with microglia, astrocytic end-feet, nerve terminals, and pericytes in the basement membrane. This cellular network prevents the passage of pathogens and toxins into the CNS, whilst contributing to the regulation of nutrient, macromolecule, and ion transport, whilst preventing the passage of pathogens and toxins into the CNS\(^2\)–\(^4\).
Tight junctions (TJs) between endothelial cells of the BBB prevent the free passage of macromolecules whilst also limiting the paracellular diffusion-mediated transport of most polar solutes\textsuperscript{5,6}. Importantly, the ability of TJs to be sensitive to factors in the CNS allows for the creation of a dynamic BBB. Examples of circulating endogenous neurotransmitters, cytokines and chemical factors able to achieve this functional modulation, include vascular endothelial growth factor (VEGF), nitric oxide (NO), glutamate, interleukin-1, and histamine\textsuperscript{7–9}. Through signalling, these factors modulate the assembly of complexes forming the TJs, thus altering the permeability and restrictions placed on paracellular diffusion. Additionally, factors such as ATP, inositol-1,4,5-triphosphate, and glutamate, can also be locally produced and released by the aforementioned associated microglia, astrocytes, neuron terminals etc. in the neurovascular unit\textsuperscript{10}. Finally, interactions at the level of the basement membrane extracellular matrix and cell-to-cell interactions add an additional layer to the regulatory capacity induced by signalling from vasoactive stimuli\textsuperscript{11}. 
Figure 2 – Barriers of the brain. Schematic depicting the cellular networks of the blood brain barrier (BBB), and the blood-cerebrospinal fluid barrier (BCSFB) at the choroid plexus (CP) within the CSF-filled ventricles. CP = choroid plexus, LV = lateral ventricles, 3V = third ventricle, 4V = fourth ventricle.

c. Blood-CSF Barrier (BCSFB)

The BCSFB, also referred to as the choroid plexus (CP), is located within the cerebral ventricles (Figure 1, Figure 2). The CP floats in the CSF and has been described to pervade the four ventricles of the brain in a fashion akin to that of a fishing net spread across water\textsuperscript{12}. Within
these ventricular compartments, the CP has a similar ultrastructure, as shown in Figure 2: a vascular network of endothelial cells is surrounded by a single layer of choroid plexus epithelial cells (CPEcs) in a ring formation\textsuperscript{13}. Importantly, unlike the BBB, the tight junctions exist between the CPEcs rather than the endothelial cells. The endothelial cells themselves differ markedly from those found at the BBB due to the presence of fenestrations and leaky inter-endothelial junctions\textsuperscript{14}. These endothelial cells facilitate the controlled passage of fluid and solutes into the interstitial fluid (ISF), which exists between the endothelial cells and CPEcs. At the basolateral membrane of CPEcs facing the ISF, there is uptake of solutes, ions, and macromolecules into the CPEcs\textsuperscript{14}.

The BCSFB has been identified to have several key physiological functions, but its most recognised function is the secretion, purification, and homeostatic regulation of cerebrospinal fluid (CSF), which contributes towards the generation of intracranial pressure. The ability of the hydrated CP to spread itself across the ventricles, its vast surface area (~50\% that of the BBB)\textsuperscript{15}, the presence of the apical brush border and large mitochondrial content within CPEcs\textsuperscript{16}, as well as the polarised expression of a variety of transporters and receptors\textsuperscript{17}, all contribute towards the highly transport-oriented role of the BCSFB-CP system. It is thought that the transport of $\text{Na}^+$, $\text{Cl}^-$ and $\text{HCO}_3^-$ from the plasma, into CPEcs, and into the ventricles from the apical surface of the CPEC, creates an osmotic gradient for the water which drives the net secretion of water across the CP, i.e., the production of CSF. However, recent evidence has emerged that highlights the importance of other mechanisms, such as co-transporters, to drive CSF secretion\textsuperscript{18}.

For decades, it was believed that the CP’s sole function was to produce CSF. However, more recent literature has highlighted the broad range of roles associated with the CP-BCSFB locus. For example, the CP serves as an integrator of circadian clock periodicity signals, as well as functioning as a signalling hub to relay information to the hypothalamus via signalling factors delivered by CSF flow\textsuperscript{19,20}. The CP tissue also secretes several factors required for CNS homeostasis, such as nerve growth factor (NGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF2)\textsuperscript{12,21}. Ultimately, the BCSFB acts as a surface for the selective exchange of nutrients, macromolecules, and various immune cells between the blood and the CSF, which contributes to creating a controlled signalling environment for
neuronal and glial function and development. As with the BBB, the function of the BCSFB is subject to modulation by circulating factors \(^{12,21}\), as well as exogenous agents, both of which are able to bind to an array of receptors and transporters at the BCSFB. For example, the BCSFB serves as a receiver of endogenous sex hormone signals (e.g., progesterone), which can modulate CPEC-mediated synthesis of and secretion of the transport protein, transthyretin (TTR) \(^{22}\). Although limited in number, research using invasive methodologies have attempted to describe the effect of various pharmacological agents on the choroid plexus-BCSFB locus. Examples include caffeine \(^{23,24}\), CO\(_2\) (hypercapnia) \(^{25-27}\), and vasopressin\(^{28,29}\). The BCSFB’s role of IGF-1 transport is coupled to Amyloid-B clearance. Upon the binding of IGF-1 to its receptor on the CPEC, signalling processes stimulates the activity of an endocytic receptor, megalin (LRP-2), which mediates the further transport of IGF-1, and the simultaneous clearance of Amyloid-B \(^{30,31}\). As described in later sections within this thesis (e.g. Chapter 1 – Section i, and Chapter 2), the administration of these substances may be able to serve as possible “challenges” to assess the vascular reactivity of the BCSFB, thereby giving an insight on barrier health (Chapter 2).

d. BCSFB in ageing

Given the BCSFB’s versatility and broad range of associated functions, the impairment of BCSFB function will likely disrupt the homeostatic balance of the CNS. From the literature, it is clear that changes in CPEcs are associated with ageing and Alzheimer’s disease (AD). It is well established that accumulation of Amyloid β in brain extracellular space is a major hallmarks of AD pathogenesis \(^{32}\). CSF and ISF within the cerebral interstitium has been shown to play a critical role in the clearance of toxic molecules and metabolic waste, such as Amyloid β, through macroscopic waste clearance mechanisms in the brain, such as the proposed glymphatic route\(^{33}\) and intramural peri-arterial drainage route \(^{34}\). In addition, CPEcs have also been shown to contain transporters which scavenge Amyloid β out of the CSF, and back into the vasculature, e.g. megalin/LRP-2, TTR \(^{35-37}\). In ageing and AD, CPEC functionality is markedly deranged, which presents as a loss of structural integrity and impairments in secretory function\(^{38-40}\). These distinct changes in CPEcs may initiate or exacerbate cascades towards neurodegenerative disease progression, for example, through impairments in downstream CSF-mediated clearance routes, or by limiting the ability of CPEcs to remove toxins from the
A study by Carro et al. conducted in mice and rats showed that in normal aged animals, the aforementioned IGF-1-coupled transporter, megalin/LRP2, is decreased, thereby also decreasing the clearance of Amyloid B. Carro et al. posited that the attenuated IGF-1/megalin transport may contribute to an increased risk of neurodegeneration. Furthermore, the TTR protein secreted by the CP has also been shown to bind Amyloid B. Transgenic mice which overexpress human amyloid precursor proteins, and are knockouts for the TTR gene (deletion), present with an accelerated Aβ deposition and toxicity. In addition, decreased level of TTR in the CSF has been reported in humans with severe dementia and in AD, further emphasising the importance of this pathway.

Ageing remains the primary risk factor for the development of dementias such as Alzheimer’s Disease, as well as other neurodegenerative conditions such as Parkinson’s disease. The increased prevalence of these age-associated disorders within our society calls for a deeper understanding of the changes which renders an ageing brain vulnerable to these pathologies.

It has become increasingly evident that the CP-BCSFB locus undergoes numerous morphological and functional changes within the ageing brain, with associated impairment of the BCSFB’s role in secretory, transport, immune, and barrier function. These changes include hypoperfusion of the CP (as suggested by BCSFB-ASL MRI by our group), impaired CSF production and turnover, changes in epithelial cell metabolism and transport, a reduction in the capacity to perform amyloid B clearance, the deposition of various species within the cell cytoplasm of the epithelial cells, and calcification of the basement membrane.

It is hypothesised that the endogenous vasoactive hormone, vasopressin, plays a role in the age-related functional impairment of the BCSFB. Significant increases in endogenous levels of vasopressin in the blood have been reported in ageing, both in humans and in animal models. At the BCSFB, vasopressin binds to V1 receptors situated on the CP epithelial cell (CPEC) membrane. Alongside the subsequent vasoconstriction to decrease CP blood flow and decrease CSF secretion, this binding event can induce a transition towards the formation of dark CP epithelial cells, observed distinctly in-vitro and in-vivo. Although the study of these dark cells has been limited, they have been shown to occur naturally in-vivo, in the adult mammalian brain of mice, dogs and humans. Previous work comparing infant to adult rats have shown increases in the number of dark cells within the BCSFB with age, linked to
the aforementioned increase in endogenous vasopressin plasma concentrations with ageing. A more prominent dark cell population modifies hemodynamics and fluid transfer across the BCSFB, i.e. decreasing both perfusion to the CP and CSF secretion rates \(^{58}\). Therefore, it is possible that the increases in endogenous vasopressin, associated with increased dark cell formation, may contribute to decreases in the reactivity, perfusion, and secretory efficiency of the BCSFB with age.

Several studies have shown the ability of CPEC transplantation to lower the cerebral amyloid burden and neuroinflammation, as well as improving neurogenesis and memory in rodent AD models \(^{59,60}\). Thus, by conducting investigations into this locus, which is heavily implicated in the onset of neurodegenerative conditions, the BCSFB may serve as a rich source of novel biomarkers and mechanistic understanding in dementia.

e. CSF metabolism, flow, and dynamics

**CSF generation at the choroid plexus**

CSF secretion at the choroid plexus is defined by the net movement of water from the plasma-ISF compartment to the ventricular CSF pool, facilitated by the CP epithelial cells at the BCSFB. Despite CP being proposed as the primary source of CSF secretion over 200 years ago, the molecular mechanisms underpinning the secretion of CSF are yet to be fully elucidated. A recent review of cerebrospinal fluid production by MacAulay et al. \(^{18}\) highlights the complexity of this secretion process, which cannot be described by the action of osmotic gradients enabling fluid to be simply drawn from vasculature into ventricular CSF. Rather, CSF secretion is a complex process that involves the coordinated action of several transporters \(^{18}\).

The first step in CSF production is the filtration of plasma from the capillaries into the extracellular space of the choroid plexus – the interstitial fluid (ISF) (Figure 2 above). Similar to filtration at the kidney, the presence of fenestrations in the capillary endothelium facilitates this filtration, i.e. the passage of small molecules and ions \(^{21,61}\). Once the plasma
has been initially filtered into the ISF, at the basolateral side of the epithelial layer, it is subsequently used to generate CSF by the CPeCs.

Studies investigating trans-epithelial osmotic gradients have shown that epithelial layers, such as the choroid plexus, are able to transport fluid independently of – and even against – an osmotic gradient. The CP epithelia does not have an appreciable osmotic gradient \(^{62,63}\), and CSF secretion is able to occur readily in instances when there is an experimentally-induced osmotic gradient which would theoretically favour vascular reabsorption of water rather than CSF secretion – an opposing directionality \(^{62-64}\). The paradoxical uphill transport of water, despite being a shared feature by epithelial layers within the body, is a phenomenon whose molecular mechanisms are not fully understood. However, recent evidence has identified the co-transport of water to be a key mechanism facilitating net secretion (see detailed description below). Furthermore, as recently discussed by Hladky and Barrand, hydrostatic pressure from the brain’s vasculature cannot account for net secretion/transport of water across the BBB or BCSFB, because the pressure gradients required to drive this flux would be instantly opposed by osmotic gradients of tissue or CSF, causing flow to be primarily in the direction which favours movement of water into the blood. Specifically, Hladky and Barrand propose that at the BCSFB, any flow due to hydrostatic pressures driving water across the barrier soon ceases unless accompanied by solute transport because water movements will inevitably modify solute concentrations \(^{65}\).

The transport of solutes across the BCSFB is mediated by ATP-driven transporters and other transport mechanisms, where water is closely coupled. The experimental demonstrations of fluid closely following solute transport in various co-transporters has further indicated that this coupling process was independent of both the direction and magnitude of the induced osmotic challenge \(^{18}\). It was then proposed that the uphill transport of fluid is energised by downhill flux of a co-transported solute, also known as co-transporter-mediated water transport. Despite the numerous lines of experimental evidence which supports this, the precise mechanisms are not yet known. The latest research has suggested a model which
relies on a hyperosmolar coupling cavity within the transport protein itself, Na⁺/K⁺/2Cl⁻ cotransporter (NKCC1) which would mediate this complex transport process 18,66.

Another key apical transporter is aquaporin-1 (AQP-1), which has been suggested to play a major role in water transport at the BCSFB. However, further complicating the view of transport mechanisms, the direct quantitative contributions of AQP-1 to CSF secretion are not entirely understood, as the deletion or absence of AQP-1 in mice and humans have had minimal impact on net fluid transport 18,67.

Overall, the process of CSF production at the choroid plexus is a highly complex and coordinated process involving various transporters and mechanisms. Despite our longstanding knowledge of CSF secretion, there is still much to be understood about the molecular mechanisms involved. Further research is needed to gain a more complete understanding of the molecular mechanisms involved in the production of CSF at the choroid plexus, as well as their relative contributions, which could have implications for the diagnosis and treatment of neurological disorders.

**CSF flow, dynamics, and clearance routes - the classical perspective**

Given that CSF is produced primarily at the BCSFB within CP tissues 21, it is important to consider the downstream flow and dynamics of the resultant CSF, as well as proposed models for CSF-mediated clearance. Classically, the flow of CSF through the human ventricular system is thought to follow a specific route. From the choroid plexus in the lateral ventricles, the CSF flows through the interventricular foramina into the third ventricle. In the third ventricle, the CSF mixes with additional CSF produced by the choroid plexus located in the floor of the third ventricle. The CSF then flows through the cerebral aqueduct into the fourth ventricle (which also contains CP tissue generating new CSF). From there, the CSF flows out of the fourth ventricle through the foramina of Luschka and Magendie and into the subarachnoid space around the brain and spinal cord 68. Thus, the CSF generated at the lateral ventricles will eventually enter the wider, interconnected reservoir of CSF which includes ventricular and extra-ventricular sources, more specifically, reabsorption into the venous blood via arachnoid granulations and nerve roots at the subarachnoid space (SAS). The CSF within this CNS-wide pool, together with its communication and mixing with the interstitial fluid (ISF) bathing cells
within the brain, forms a major role of the clearance of accumulating metabolic solutes, waste and neurotoxins.

**Glymphatics and IPAD**

In the rest of the body, i.e. outside of the CNS, a lymphatic system is used to transport these metabolites into the lymph nodes to process and clear metabolites. Lymphatic drainage is essential for maintenance of overall tissue water and solute balance, homeostasis, metabolism, and immunity. It had been widely accepted that the CNS was devoid of a lymphatic system, as their existence had not been observed. However, research from the last decade has brought to light that there is uncertainty regarding the existence of a ‘conventional’ lymphatic system within the brain. Rather, the CNS of rodents and humans have been shown to contain unique lymphatic drainage structures which form the meningeal (dural) lymphatic system.

The glymphatic system, which takes its name from the glial cells and the proposed similarity of its ‘clearance’ functions to the lymphatic system, is a recently proposed macroscopic waste clearance system. It is composed of a network of perivascular channels formed by astroglial cells, which surround the arterioles, capillaries and venules, thereby enabling the clearance of interstitial solutes and wastes. First, there is inflow of some CSF into these perivascular channels (periarterial) from the SAS. This periarterial influx of CSF is followed by the exchange of CSF into the tissues, more specifically, the transport of water via aquaporin-4 (AQP-4) localised to astroglial endfeet. Once the CSF has entered the tissues and is bathing the cells, it can be considered ISF which is used as a medium to mix with the metabolic waste and solutes to ‘wash’ the tissues. Finally, there is egress of the waste-filled ISF via perivenous spaces.

Aside from the glymphatic system, an alternative hypothesised model is the intramural periartrial drainage (IPAD) pathway. At the cerebral vasculature, the IPAD pathway has been proposed to function between layers of smooth muscle cells (SMCs) and basement membranes (BM). Waste products, such as amyloid-B, are able to enter the ISF and move intramurally (i.e. between the SMC and BM layers) and in counter-flow towards cervical
lymph nodes. Using the intrinsic contractile forces of the SMCs, the ISF containing the waste products are able to be transported out of the brain.

**Brain fluid dynamics in rodent vs human brains**

There are several points of difference in anatomy and physiology between rodents and humans which must be considered when extending the interpretation of CSF flow, dynamics, and clearance, as well as the more upstream role of the BCSFB focussed on within this thesis. The flow of CSF in the rodent brain, e.g. mice and rats, is somewhat similar to that in humans. Rodents also have four ventricles, but the size and shape of the ventricles are different from those in humans. In rodents, the lateral ventricles are larger (relative to the volume of the whole brain) and more elongated, while the third and fourth ventricles are smaller in size. In addition, the choroid plexus tissue within the ventricles was found to be relatively smaller in rodents than in humans.

Furthermore, the rodent brain is at a much smaller scale to that of the human brain, i.e. 10 mm vs 140 mm wide, and combined with its less physically convoluted/curved nature, will impact the extent to which advection (i.e. delivery via bulk flow of the CSF) will impact flow dynamics. Additionally, the metabolic demands of the brain in rodents are different from those in humans. Rodents have a higher metabolic rate compared to humans, which influences the production and flow of CSF. Given the increased rate at which nutrients are required to be supplied to metabolically active tissues, there will be a corresponding rate at which metabolic waste also needs to be cleared by CSF. As such, there are also differences in CSF turnover rates between species, i.e. the rate at which CSF is produced and absorbed.

In some species, such as rats, CSF turnover is higher than in humans. This difference is, in part, due to physiological differences in cardiovascular function which contribute to CSF dynamics. For example, a critical point of difference is cardiovascular function: the mouse heart beats at approximately 600 beats/min and the human at approximately 60 beats/min. As CSF motility is known to be driven by cardiac pulsation, this will have a significant impact on CSF dynamics in the rodent vs human brain. In several mammalian species that have been used for the study of the CP-BCSFB-CSF system, e.g. humans, Rhesus monkeys, goats, sheep, dogs, cats, rabbits and rats, it has been observed the rate of CSF secretion occurs...
at similar rates per unit of choroidal weight, which scales approximately linearly with total brain weight

In the classical model of CSF flow in humans, arachnoid projections (granulations and villi) in the skull are considered to main site of CSF outflow. However, to date, evidence detailing the presence of arachnoid granulations in the mouse brain is scarce. As discussed by Proulx, granulations are evident to the naked eye, and as such, they have only been described in larger animals, and not in rodents. Therefore, it is believed that in rodents devoid of these granulations, the meningeal lymphatic system (discussed above), is believed to play a bigger role in CSF outflow.

Overall, while the basic route of CSF flow is similar in humans and rodents, there are some notable differences in the size and shape of the ventricles, rates of CSF turnover, cardiovascular driving forces, metabolic demands, and the distribution of components needed for CSF-mediated clearance routes.

### f. Investigations into choroid plexus function

**Invasive and ex-vivo tools: microspheres**

Despite the importance of the BCSFB in both physiology and pathology, and its high potential for providing novel biomarkers, this region remains relatively understudied compared to the BBB. The lack of research dedicated to understanding the BCBSF’s role has, in part, stemmed from the absence of non-invasive techniques for functional assessment. Previous pre-clinical studies measuring choroid plexus blood flow were conducted using highly invasive approaches: intravascular delivery of radioactive microspheres or tracers alongside terminal surgical procedures – e.g. femoral artery catheterization and cardiac atrial catheterization following a thoracotomy. Aside from being impossible in the clinical setting, these earlier approaches were also limited in their temporal resolution and typically provided single time point blood flow measurements per subject. In the 1980s, Faraci et al. applied a microsphere approach to investigate the choroid plexus’ ability to have its blood flow modulated by pharmacological agents. For example, in anaesthetized and awake rabbits, the infusion of arginine vasopressin evoked an exclusive decrease in choroid plexus perfusion, with no
measurable effect on perfusion to the cortex\textsuperscript{90}. Subsequent studies highlighted that serotonin elevated CP perfusion in monkeys and dogs, with minimal effects on cerebral blood flow\textsuperscript{91}. Together, these studies utilising highly invasive methodologies highlighted the ability of endogenous signalling factors to regulate perfusion to the choroid plexus.

**CSF secretion measurements**

Functional assessment of the choroid plexus subsequently transitioned towards using rates of CSF secretion – the primary function of the CP – as an indirect readout of CP function. For example, Chen et al. showed that CSF production rates were reduced in aged sheep. CSF secretion rates were determined in an invasive manner using isolated and perfused CP tissue from sheep brains\textsuperscript{92}. Furthermore, several studies have demonstrated that CSF secretion rates are reduced in the human brain during ageing and Alzheimer’s disease\textsuperscript{38,40}. Again, studies of this nature necessitated the use of the invasive surgical approaches, such as the Masserman method which aimed to quantify the time required for CSF pressure to normalise following a lumbar puncture for CSF drainage\textsuperscript{93}. Alternatively, the Pappenheimer methodology, also known as the ventriculo-cisternal perfusion method, was applied in several animal models to quantify CSF production by cannulation, infusion of a tracer, and the subsequent quantification of tracer dilution in the perfusate, as a surrogate marker of CSF production\textsuperscript{94,95}.

These approaches are limited not only by the invasive nature of the methodologies used, but also by the high levels of variability in the measured result dependent on the precise methodology used. For example, the validity of results obtained using the Masserman technique has been criticized, as the method assumes that neither formation nor absorption rates are changed by alterations in pressure. The pressure, however, is likely to be changed due to the lumbar puncture required to access the CSF in the spine\textsuperscript{96}. The Pappenheimer technique, also suffered from an important limitation: the procedure was difficult to apply in clinical settings because of its invasiveness. The hour-long infusion necessitated a ventricular and extraventricular CSF catheter. Furthermore, both the infusion rate and infused volume exceeded the physiological range of CSF flow\textsuperscript{96}. The quantified rates of secretion provided by the Pappenheimer technique have also been criticised due to potential confounding factors, such as the escape of indicators or water into the brain tissue\textsuperscript{97}. 

Pharmacological studies of the BCSFB

Vasopressin, also known as anti-diuretic hormone (ADH), is a vasoactive, endogenous hormone which binds to the CPeC-bound V1 receptor. Evidence from invasive microsphere measures, as well as BCSFB-ASL in our lab, has highlighted the ability of vasopressin administration to cause vasoconstriction at the BCSFB\(^1,28\). CSF secretion measurements in rabbits have also shown that vasopressin is able to downregulate CSF secretion at the choroid plexus\(^28,29\).

Caffeine, an adenosine receptor antagonist, has also been reported to affect CSF production, both acutely (decreased CSF production) and chronically (increased CSF production). A study by Han et al. provided evidence to support the involvement of choroid plexus-bound A\(_1\) adenosine receptors in the caffeine-mediated alteration of CSF.\(^23\) Furthermore, adenosine itself has been shown to increase CP BF.\(^24\)

CO\(_2\)-driven dilation of the cerebral vasculature is an established observation, which forms the basis for hypercapnia-based cerebrovascular reactivity paradigms.\(^98\)–\(^101\) However, there is limited and conflicting literature describing the effects of hypercapnia on the vasculature associated with the choroid plexus, with animal models displaying increases, decreases, or no change in perfusion to the choroid plexus following hypercapnia.\(^25\)–\(^27\).

MRI tools to study the choroid plexus in-vivo

More recently, the study in human subjects by Bouzerar et al. reported relative changes to perfusion at the CP and permeability of the vasculature at this locus using gadolinium-based contrast agents (GBCA) for dynamic susceptibility contrast MRI (DSC-MRI).\(^102\) The use of an intravenous GBCA is limited by concerns of tissue deposition of these contrast agents within subjects.\(^103\) In addition, gadolinium has been shown to be nephrotoxic in patients with pre-existing kidney conditions, such as chronic renal insufficiency and renal failure.\(^104,105\) These concerns, combined with DSC-MRI being able to provide data only at a single time point, limits its clinical appeal and scope of application to study dynamic changes at the BCSFB.

Arterial spin labelling (ASL) MRI is the “gold standard”, non-invasive methodology used to quantify perfusion. ASL MRI negates the requirement of injectable tracers or contrast agents.
by utilising endogenous water as a tracer and does not require terminal experimental end points. Capitalising on these advantages that an ASL-based approach holds, recent work by Evans et al. describes a novel, translational ASL-based approach for the measurement of BCSFB function by quantifying the rate of delivery of arterial blood water across the BCSFB, into ventricular CSF. This technique – henceforth referred to as “BCSFB-ASL” – utilises an ultra-long, 220 ms echo time (at 9.4T). To obtain a surrogate index of BCSFB function, the integral of the ASL signal at TE = 220 ms is taken across a large imaging volume positioned at the caudal lateral ventricles, where the choroid plexus has been shown to predominantly occupy. Evans et al. characterised the methodology, and subsequently applied the technique in the mouse brain with further proof of application in the human brain. Of note, the application in the mouse brain reproduced findings from invasive studies by Faraci et al., whereby the application of a vasopressin pharmacological challenge in adult mice produced an exclusive decrease in BCSFB-mediated water delivery into ventricular CSF, and thereby a decrease in BCSFB function, relative to a vehicle. In addition, the technique was applied to aged mice where a marked decrease in rates of BCSFB-mediated labelled water delivery was detected relative to adult controls.

**g. ASL background**

The functional MRI approach implemented in this work for the non-invasive measurement of BCSFB-mediated water delivery rates and cortical cerebral blood flow (CBF), is based on arterial spin labelling (ASL) MRI. ASL is a technique developed to assess perfusion, with its first application in the rat brain by Detre et al. in 1992. ASL has since been used to predominantly measure cerebral perfusion, with further adaptations to become sensitive to detecting and characterising more specific neurofluid and hemodynamic processes, such as BCSFB-ASL for the quantification of BCSFB-mediated water delivery rates as a surrogate index for BCSFB function.

The general principle of ASL is to label a bolus of intravascular blood water, and subsequently quantify the rate of the delivery of the tagged bolus to the tissue as a quantitative estimate of tissue perfusion. Inflowing tagged water molecules have a reduced longitudinal magnetization causing a subsequent reduction in the longitudinal magnetization of the tissue.
by approximately 1%, thus creating a labelled image. By subtracting this labelled image from a control image, a perfusion-weighted ASL image will be produced where the difference in signal (control – labelled) is proportional to blood flow\textsuperscript{108,111}.

A major advantage of ASL is that the labelled endogenous water acts as a tracer which is actively and passively transported, thus eliminating the need for an exogenous tracer such as gadolinium-based contrast agents (GBCA) which have been shown to be associated with nephrotoxicity and tissue deposition. Additionally, techniques which exploit the paramagnetic properties of deoxy-haemoglobin, such as fMRI-BOLD, are sensitive to local blood oxygenation and can provide information on perfusion. However, unlike ASL, they do not provide direct measures of quantitative blood flow due to dependence on multiple parameters (absolute blood volume, blood volume, flow changes, vessel size etc.)\textsuperscript{112,113}.

The original ASL method for labelling arterial spins was proposed by Williams \textit{et al.}\textsuperscript{114}. There are currently three main ASL techniques, defined by different labelling strategies: pulsed ASL (PASL), continuous ASL (CASL) and pseudo-continuous ASL (pCASL)\textsuperscript{109,115,116}. One of the earliest ASL technique developed for application in the human brain was CASL, which uses continuous RF-irradiation to tag blood water as it flows through a narrow labelling plane, often positioned across multiple feeding arteries upstream from the imaging region\textsuperscript{108,110}. A labelled image is produced by applying inversion pulse(s) across these upstream feeding arteries, distal to the imaging slice. A control image is obtained by not applying any labelling to the vessels, and instead is applied above the head to produce identical magnetisation transfer (MT) effects without labelling arterial blood. With CASL, a post-labelling delay (PLD) is selected after a given labelling duration to allow the tagged spins to reach the imaging region. Despite providing a signal with a high signal-to-noise ratio (SNR), these sequences were often difficult to implement and suffered from heating concerns resulting from the continuous irradiation.

PASL employs a single radiofrequency (RF) pulse to perform a slice-selective (labelled) and a global (control) inversion of the spins, without the use of continuous RF irradiation. The inflow time (TI) is selected as the time between the labelling pulse and the image acquisition. PASL sequences apply labelling across wider planes, in a manner which can be asymmetrical,
or symmetrical (e.g. FAIR-PASL\textsuperscript{117}, Figure 3a) in nature, with an inflow time (TI) being selected between labelling and image acquisition\textsuperscript{118}. Of note, ASL images acquired at a single inflow time (TI) value are displayed in Figure 3. Single-TI ASL values have been chosen here to reflect the delivery of labelled water into the brain tissue for standard-ASL, or into ventricular CSF with BCSFB-ASL. Previous measures using multiple TIs by Evans \textit{et al.} have informed on the TI at which the ASL signal is at a maximum (see Figure 5a below)\textsuperscript{1}. The work presented in this thesis utilises both single-TI and multi-TI approaches. Due to the pulsed nature of the irradiation, there are reduced heating effects on tissues. However, PASL data is often acquired with a lower SNR relative to CASL. pCASL – a hybrid between PASL and CASL - uses a series of short RF pulses to label spins in a narrow labelling plane, comparable to the labelling planes used in CASL. As the “pseudo-continuous” nature suggests, this train of short pulses mimic the action of a single, continuous pulse. Again, a PLD is selected between labelling and image acquisition. pCASL utilises the higher SNR associated with CASL, with the lower heating effects associated with PASL, and the added benefit of being more compatible with standard hardware available on clinical scanners when compared to CASL\textsuperscript{116,119}.

To measure changes in BCSFB function (BCSFB-ASL) and cerebral blood flow (standard-ASL) in a non-invasive manner, the work herein implements PASL using a flow-sensitive alternating inversion recovery (FAIR) sequence with a labelling strategy shown in Figure 3, which has been previously shown to give reproducible labelling efficiency\textsuperscript{120}. The FAIR technique alternates between a global inversion (non-selective) and a slice-selective inversion\textsuperscript{117}. The slice-selective inversion (labelled images) inverts spins within a specified labelling slab around the imaging slice. The global, non-selective inversion (control images) uses the same inversion pulse but without the slice-selection gradient, thus inverting spins within the entire sensitive volume of the RF coil, across a larger volume of the subject. Subtraction of the control image from the labelled image results in a perfusion-weighted ASL image that reflects the signal from the labelled blood water that has moved into the imaging slice in the given inflow time (TI). Figure 3a displays a conventional FAIR-PASL scheme to quantify CBF, whereas Figure 3b depicts the specific labelling scheme used for BCSFB-ASL, which aims to account for CSF motion effects by inverting all CSF spaces within the brain identically in both the global (pulse
width covers whole subject) and slice-selective (pulse width covers brain, 20 mm) acquisitions.

**Figure 3 - FAIR PASL labelling schemes.** a) standard ASL (conventional FAIR-PASL scheme), b) BCSFB-ASL (adapted FAIR-PASL scheme). Schematic displays the imaging slice (green) and inversion slab (orange) overlaid onto a T2-weighted, sagittal image of the mouse brain, for control/non-selective and labelled/slice-selective labelling schemes. Under these images, representative, coronal ASL images (control, labelled, and ΔM) are shown at TE = 20 ms for standard-ASL (a), or TE = 220 ms for BCSFB-ASL (b). Panels on the right display a T2-weighted, coronal image of the mouse brain for comparison.
h. Theory behind BCSFB-ASL

The echo time (TE) parameter in MRI refers to the time between the application of the RF excitation pulse and when the MRI signal is received, where the image is captured using either a spin echo or gradient echo. When choosing the TE parameter for ASL measurements, it is important to choose a value which preserves the signal from the transverse magnetization decay (Figure 4), e.g. 20 ms at 9.4T for the measurement of cortical CBF using traditional-ASL in the brain. BCSFB-ASL utilizes an ultra-long, 220 ms echo time acquisition (at 9.4T), which ensures that the signal present in the functional images is captured only from CSF compartments which have a long T2. Assuming the T2 values of grey matter blood (30 ms\textsuperscript{121–123}), tissue (38 ms\textsuperscript{59}), and ventricular CSF (300 ms\textsuperscript{124}), using an ultra-long 220 ms TE will capture approximately 0.07%, 0.3%, and 48%, respectively, of the theoretical magnetization at TE = 0 ms\textsuperscript{1}. Hence, at this echo time, the signal arising from the grey matter and blood will be nulled due to their significantly shorter T2. This difference in transverse magnetization ($M_{xy}$) of different tissues has been depicted in Figure 4, which displays control ASL MR images alongside theoretical $M_{xy}$ data resulting from T2 relaxation following the application of a radiofrequency pulse during imaging.

As with traditional-PASL described above, BCSFB-ASL labels arterial blood water from vessels feeding the brain, and after a given TI, an image is acquired. The use of a flow-sensitive alternating inversion recovery (FAIR) labelling scheme provides a labelled image (slice selective inversion) and a control image (global inversion) which, when subtracted, produces a perfusion weighted image that importantly consists of signal arising from blood water that has travelled via the BCSFB into the CSF of the lateral ventricles (Figure 3b).
Figure 4 – Transverse magnetization (T2) decay curves at 9.4T. Simulated decay curves of the relative transverse magnetization ($M_{xy}$) for different tissues (blood, parenchymal tissue, and CSF), incorporating their respective T2 values taken from Lee et al., 2022 \textsuperscript{124} and Wells et al., 2013 \textsuperscript{123}, and Ohene et al.\textsuperscript{121,122}. T2 equation: $[M_{xy} = M_0 \exp(-t/T2)]$, where $M_0$ is the equilibrium transverse magnetization. At 220 ms, signal from labelled water in parenchymal tissues and blood will be nulled, whilst CSF spaces remain visible for our BCSFB-ASL measurement. Representative control ASL images ($M_c$) are displayed above the corresponding TE values used (position: bregma anterior-posterior -0.7 mm).

Besides the use of an ultra-long, 220 ms TE, another key feature of the BCSFB-ASL methodology is the use of a low-resolution readout, e.g., FOV = 20 mm $\times$ 20 mm, matrix = 32 $\times$ 32, slice thickness = 2.4 mm, as observed in the dataset displayed in Figure 3b. Importantly, as a result of the larger voxel size used, the low spatial resolution permits an increased sensitivity to relatively small ASL signals, i.e. the detection of labelled arterial water which has crossed via the BCSFB into ventricular CSF to become MRI visible at a given inflow time (TI). Partial volume effects (PVE) can often confound imaging conducted with low spatial resolution. Due to the use of an ultra-long TE to null signal from water in parenchymal tissue
and blood, whilst preserving signal from CSF, the low spatial readout is acceptable given the minimal PVE contributions from parenchymal tissue and blood\(^1\).

With the combination of an ultra-long TE and a low spatial resolution readout, BCSFB-ASL is able to provide a reliable measure of labelled arterial water crossing the BCSFB into ventricular CSF. By taking an integral of the BCSFB-ASL signal across the imaging volume, the data can then be quantified to provide a surrogate, non-invasive, measure of BCSFB function within the lateral ventricles. Many studies have shown that in the case of standard-ASL, measurement are taken when a large fraction of water has crossed the BBB, from the intravascular compartment, into extravascular tissues. For example, at an inflow time of 1.5 s, 70-86\% of the labelled water had been extracted across the BBB\(^{122,123,125}\). However, it is well recognised that with standard-ASL, presence of extravascular labelled blood water does not reflect a putative net secretion of water at the BBB. This is analogous to the BCSFB, where the BCSFB-ASL measurement similarly reflects the total water delivery from the plasma-ISF compartment into the ventricular CSF compartment, and as such does not reflect the net secretion of CSF across the BCSFB. Thus, our measure reflects the rate of perfusion of the CP convolved with its permeability to labelled blood water, and the mass of the choroid plexus tissue\(^1\). The more permeable the BCSFB, and the greater the perfusion to the choroid plexus, the greater the rate of total water delivery into ventricular CSF.

\textbf{i. BCSFB-ASL validation}

Several sets of data were collected by Evans \textit{et al.} which provided comprehensive evidence validating the use of an ultra-long TE-based ASL method (BCSFB-ASL) as a non-invasive surrogate measure of BCSFB function (Figure 1 from Evans \textit{et al}, adapted here in Figure 5)\(^3\).
**Figure 5 - BCSFB-ASL validation experiments.** Adapted from Evans et al. Figure 1.

a) Above—the normalised standard-ASL signal that serves (taken from a cortical ROI, TE = 20 ms) and novel BCSFB-ASL (TE = 220 ms) signal, as a function of TI (n = 12). Error bars: ±SEM. Below—example ASL images from a single mouse at increasing TI for both standard-ASL and BCSFB-ASL.

b) Normalised BCSFB-ASL signal (TI = 4 s) at the rostral and caudal section of the lateral ventricles. Example histological sections from rostral and caudal slices are shown for a mouse that underwent imaging. Error bars: ±SEM (n = 10), ***p = 0.000000014 from a one-tailed t-test.

c) The pseudo-diffusion coefficient ($D_{app}$) of the standard ASL and control signal (grey box) and the BCSFB-ASL and control signal at three inflow times (TIs). Error bars: ±SEM (n = 4). **p = 0.008 from a one-tailed t-test.
d) Schematic illustrating modulation of arterial blood water labelling efficiency, the experimental strategy used for the data presented in e and f.

e) Measured normalised BBB (1st column) and BCSFB (2nd column) ASL signal as a function of TI with full (black line) and partial (grey line) labelling efficiency. Error bars: ±SEM (n = 5).

f) The percentage decrease in labelling efficiency for the BBB (y-axis) and BCSFB (x-axis) ASL signal across the five mice (p = 0.02, Pearson’s correlation analysis, two sided).

g) phMRI: reactivity to vasopressin (100 µU/ml, I.P 0.1 ml) or saline control (I.P. 0.1 ml) challenge in adult mice. Right column: standard ASL signal probing BBB function; left column: BCSFB-ASL signal (relative ASL signal, % from baseline). Error bars: ±SEM (n = [4 vasopressin], [5 saline]).

Firstly, the dynamic time courses of the subtracted signal from both BCSFB-ASL and traditional-ASL were compared, i.e. ΔM as a function of TI (Figure 5a). The time course from BCSFB-ASL data was shown to be distinct from the time courses provided by traditional-ASL. This difference is partly due to two factors: an increased bolus arrival time (Δt), and a decreased rate of T1 decay of labelled water delivered to the CSF. The increased Δt is attributed to the differences in detectable signal: the ultra-long TE used in BCSFB-ASL permits the detection of signal only from water which crosses the BCSFB into the CSF, and not from blood water in the vascular compartment of the vascular tree at the imaging volume, which is however detected when using a shorter-TE in standard-ASL (Figure 4: transverse decay curves at 9.4T, Figure 9: 2-compartment Buxton model).

The location of the CP was determined through histological assessment of the studied mice (Figure 5b). This provided evidence to show that the BCSFB-ASL functional signal was co-localised with the CP position within the ventricles. In line with the literature detailing that the CP is predominantly localised to the caudal end of the lateral ventricles, this BCSFB-ASL signal and CP density was shown to be significantly higher at the caudal end of the lateral ventricles, as opposed to the rostral end (Figure 5b). Furthermore, when adjusted to account for the total mass of the CP within the lateral ventricles, the absolute CP BF values were consistent with previous invasive measures.
Experiments were conducted towards the aim of confirming the source of the BCSFB-ASL signal. An important condition underlying the BCSFB-ASL technique is that BCSFB-ASL signal represents labelled blood water delivered to ventricular CSF, without confounding signal contribution from labelled water in the vasculature. To ensure that this contamination was not present, a hybrid diffusion-ASL MRI method was employed to measure the pseudo-diffusion coefficient ($D_{app}$) of the ASL signal (Figure 5c) – an estimated parameter which is highly sensitive to the contribution from intra-vascular water signal\textsuperscript{126}. The similarity of $D_{app}$ values for CSF and the BCSFB-ASL signal at three separate TI values provided evidence that the signal arises from labelled water in ventricular CSF with negligible intravascular spin contamination. On the other hand, as expected, the $D_{app}$ value for traditional-ASL measuring parenchymal CBF was shown to be significantly higher than the $D_{app}$ of the parenchymal tissue itself, owing to the contribution from intravascular spins\textsuperscript{127,128}.

Steps were taken to determine that CSF movement within the ventricles was not confounding the BCSFB-ASL measurements. Decreases in labelling efficiency led to subsequent decreases in signal from both traditional- and BCSFB-ASL in a highly similar fashion (Figure 5d-f). This highlights that both signals derive from blood water which has been labelled in the brain’s feeding arteries, which is then delivered either to the parenchymal tissue (traditional-ASL measurement) or the ventricular CSF (BCSFB-ASL measurement), without the latter measurement being reflective of CSF movement.

Demonstrating the specificity of this method as a correlate of in-vivo BCSFB function, Evans \textit{et al.} administered a pharmacological vasopressin challenge in the anaesthetized mouse brain (Figure 5g). In line with previous work using the invasive microsphere technique, vasopressin was shown to evoke exclusive decreases in water delivery across the CP, with no measurable CBF change\textsuperscript{28}. Complementing this data, the administration of a saline vehicle resulted in no changes recorded for CP BF or CBF\textsuperscript{1}.
### j. Investigating CP function: traditional ASL

More recently, several papers have used a traditional ASL approach (as used to investigate CBF) to quantify perfusion to the choroid plexus in the human brain\textsuperscript{129–131}. For example, Johnson et al. assessed CP perfusion in the context of neoangiogenesis in patients with intracranial stenosis\textsuperscript{130}. pCASL data acquired at a single PLD (post-labelling delay, i.e. time between labelling and image acquisition) were co-registered to subject-wise anatomical images, as well as a brain atlas, in order to selectively extract CP perfusion values\textsuperscript{130}. Further work by Johnson et al. applied a similar methodology, i.e. pCASL with T1-weighted image coregistration, to assess CP perfusion in Sickle Cell Disease and Moyamoya vasculopathy patients\textsuperscript{131}.

Zhao et al. sought to develop a methodology for characterising CP perfusion dynamics in humans, by acquiring pCASL data at selected combinations of labelling durations (LDS) and post-labelling delays (PLDs). As with the work conducted by Johnson et al., ASL images and proton-density images were co-registered to segmented, T1-weighted anatomical images to more specifically isolate perfusion to the CP tissue\textsuperscript{129}.

Importantly, these three studies highlight that due to the inherently low resolution of ASL and the branching, coral-like structure of the choroid plexus tissue, perfusion measures of the choroid plexus will be impacted by partial volume effects (PVE). ASL voxels selected with an ROI will therefore contain a mixture of signal contributions from various tissues: desired choroid plexus tissue, CSF (with zero perfusion), and periventricular grey/white matter tissues. PVE issues are also compounded with the limited quality of co-registration between lower resolution ASL images and T1-weighted structural images\textsuperscript{129–131}. Although studies utilising traditional ASL approaches may be promising in the human brain to investigate apparent CP perfusion in healthy and altered brain states, the application of a similar approach is challenging in the mouse brain, where there will inevitably be much more pronounced partial volume contributions within ASL voxels due to the differences in brain size. As highlighted prior, by using an ultra-long TE to isolate signal from CSF, in conjunction with a low-resolution readout, BCSFB-ASL is able to minimise issues arising from PVE.
**k. Investigating CP function: ultra-long TE ASL**

Several studies have used an ultra-long TE ASL MRI acquisition in a similar manner to the BCSFB-ASL methodology described by Evans et al., which was designed to enable the isolation of labelled water crossing the BCSFB into ventricular CSF. For example, Lee et al. applied a CASL approach in the rat brain to quantify blood to CSF barrier function and choroid plexus tissue perfusion. This work quantified three parameters to describe the dynamics of labelled water delivery and transport at the BCSFB locus: a) water delivery across the BCSFB water calculated directly from CASL measurements using an ultra-long TE (similar to BCSFB-ASL), b) CP perfusion calculated directly from CASL measurements using a short TE (similar to using standard-ASL for assessing CP perfusion), c) apparent CP perfusion, which is modelled to account for the mixing of signals from a) and b). Importantly, T2 relaxometry was used to identify CP compartments for the analysis, i.e. tissue and CSF partial volumes. Importantly, using a higher resolution readout than the application of BCSFB-ASL in the mouse brain (in plane resolution = [mouse: 0.63 mm/voxel] vs [rat: 0.45 mm/voxel], slice thickness = [mouse: 2.4 mm] vs [rat: 1.0 mm]), this study was able to demonstrate that BCSBF-ASL functional hotspots were co-localised to the locations of CP tissue within the lateral ventricles. This provides further confidence that BCSFB-ASL, and the use of an ultra-long TE in particular, is sensitive to BCSFB mediated water exchange occurring at the CP.

In the human brain, Petitclerc et al. demonstrated the application of a multi-PLD pCASL methodology protocol with a series of ultra-long TEs to characterise the locations and dynamics of blood-CSF water exchange. Here, the combined multi-PLD and multi-TE dataset were modelled to describe the dynamic exchange of labelled water from blood to CSF. As expected, labelled water transport was detected in the CSF proximal to the choroid plexus. Interestingly however, this study revealed that the ultra-long TE ASL signal is distributed in regions beyond that of the lateral ventricles, namely the subarachnoid space (SAS). The potential for this finding to reflect blood-CSF water exchange sites beyond that of the BCSFB is investigated in Chapter 4 of this thesis.
1. **Standard-ASL and BCSFB-ASL quantification**

**Image processing and analysis for blood flow quantification**

The work presented in this thesis will quantify cortical CBF with standard-ASL, and rates of BCSFB-mediated water delivery into ventricular CSF with BCSFB-ASL. In order to obtain absolute values of these rates, the acquired ASL-MRI images undergo processing and are used to fit several models (for example, see Chapter 1 Figure 5a). Cortical CBF was quantified using the general Buxton kinetic model which was fit to data acquired at TE = 20 ms\textsuperscript{133}: Delivery of labelled blood water to ventricular CSF from BCSFB-ASL images (TE = 220 ms) was quantified using an adaptation of the 2-compartment Buxton model\textsuperscript{1,125,134}. The Buxton kinetic modelling approaches used within this thesis require several input parameters from the subject-wise ASL data within a selected ROI (either the cortex for standard-ASL, or lateral ventricles for BCSFB-ASL): global image magnetisation (“control”, $M_C$), slice-selective image magnetisation (“labelled”, $M_L$), and the $\Delta M$, i.e. the difference between $M_C$ and $M_L$. These data are acquired as a function of inflow time (TI), henceforth referred to as “multi-TI data”. The analytical pipelines for multi-TI ASL data are summarised below: standard-ASL (Figure 8) and BCSFB-ASL (Figure 9). Prior to demonstrating the analytical process for the ASL images acquired, I will first describe the Buxton kinetic models we use.

**Buxton model fitting to calculate CBF**

Cerebral blood flow (CBF) was quantified by taking the standard-ASL signal, i.e. $\Delta M$ values, and applying the general Buxton model (single compartment model)\textsuperscript{133}, as described by Equation 3 and Figure 6 below. For the single compartment, general model, the initial baseline at $0 < TI < \Delta t$ (the arterial transit time) reflects the arrival time of the tagged bolus of water. Until the arterial transit time, $\Delta t$, the signal will initially be zero until the leading edge of labelled water (with temporal length $\tau$) has arrived in the imaging volume (Figure xxx a). When the labelled bolus arrives at the cortical ‘tissue’ (in this case a combination of intravascular (IV) and extravascular (EV) components (Figure 6b)), there will be subsequent delivery of the labelled bolus into the tissue compartment at times $\Delta t < TI < \tau + \Delta t$. Upon flowing into the cortical ‘tissue’ compartment ($\tau + \Delta t < TI$) the labelled water magnetization will decay with the relaxation time of the cortical tissue ($T_{1\text{cortex}}$).
<table>
<thead>
<tr>
<th>TI range</th>
<th>ASL signal ($\Delta M$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0 &lt; TI &lt; \Delta t$</td>
<td>$\Delta M = 0$</td>
</tr>
<tr>
<td>$\Delta t &lt; TI &lt; \tau + \Delta t$</td>
<td>$\Delta M = 2. M0. (TI - \Delta t). \alpha. f. \left( \exp \left( -\frac{TI}{T1b} \right) \cdot q_1(TI) \right)$</td>
</tr>
<tr>
<td>$\tau + \Delta t &lt; TI$</td>
<td>$\Delta M = 2. M0. \tau. f. \alpha. \left( \exp \left( -\frac{TI}{T1b} \right) \cdot q_2(TI) \right)$</td>
</tr>
</tbody>
</table>

$$
q_1 = \frac{e^{k(TI)}(e^{-k\cdot\Delta t} - e^{-k(TI)})}{k(TI - \Delta t)}
$$

$$
q_2 = \frac{e^{k(TI)}(e^{-k\cdot\Delta t} - e^{-k(\tau + \Delta t)})}{k\tau}
$$

$$
k = \frac{1}{T1b} - \frac{1}{T1_{cortex} + f/\lambda}
$$

**Equation 3 - Buxton general kinetic model for quantification of absolute cortical CBF using standard-ASL data (low TE values, e.g. 20 ms).** Model fitting output parameters are shown in red ($f$, $\Delta t$, $\tau$).
Figure 6 – General, single-compartment Buxton model. A single, ‘tissue’ compartment is defined, which combines intravascular (IV) and extravascular (EV) contributions. Δt is defined as the arterial transit time, i.e. the time for the labelled bolus of water with temporal length (τ) to arrive at the ‘tissue’ compartment within the imaging slice. BBB = blood brain barrier.
Transit time values are taken from Evans et al.\textsuperscript{1}. For clarity, the inversion plane shown here in green is more specifically used for CASL. For FAIR PASL, a much larger inversion plane is used, as depicted in Figure 3 (FAIR ASL labelling schemes). a) Prior to the arrival of the labelled bolus of water at the tissue (0 < TI < Δt), the labelled bolus is present in the proximal vessels. b) When the labelled bolus is present within the tissue compartment (Δt < TI < τ + Δt), there is delivery of the labelled water across the BBB to perfuse tissues, \textit{i.e.} ‘flow’\textsuperscript{133}. Vessel component of diagram created with Biorender.com.

Several key inputs are required for this model: ΔM (the subtracted ASL signal, M\textsubscript{L} - M\textsubscript{c}), M\textsubscript{0} (the equilibrium longitudinal magnetization), and T\textsubscript{1\textsubscript{cortex}} (the longitudinal relaxation constant for the cortex). The calculation of these parameters will be described later (see equations 5 and 6 below). There are also terms which describe the inversion efficiency (α = 0.9), the equilibrium tissue/blood partition coefficient of water (λ = 0.9\textsuperscript{133}), and the longitudinal relaxation of arterial blood (T\textsubscript{1\textsubscript{b}} = 2.5 s, from the literature\textsuperscript{135}). λ = ρ\textsubscript{t} / ρ\textsubscript{b}, which is the density of water in tissue divided the density of water in the blood\textsuperscript{136,137}.

ΔM is proportional to flow, f, which in this model is described as the volume of arterial blood delivered to a voxel volume per second \textit{– i.e.} water delivery to brain tissue. Thus, for standard-ASL, the extracted flow is the rate of delivery of labelled blood water to cerebral tissue. The Buxton model can be rearranged to calculate flow from the ΔM values obtained. Furthermore, fitting this model to ΔM signal also permits the extraction of arterial transit time, Δt, and the temporal length of the tagged bolus, τ. Arterial transit time describes the time taken for the leading edge of the tagged bolus to appear in the imaging voxels. For the CBF measurements collected within this body of work, all three parameters (f, Δt, and τ) are extracted.

**Buxton model fitting to calculate rates of BCSFB-mediated water delivery**

Rates of delivery of labelled blood water to ventricular CSF from BCSFB-ASL images (TE = 220 ms) were quantified using the 2-compartment perfusion model. This 2-compartment model was first described by Alsop and Detre\textsuperscript{134}, and then later adapted by Wong \textit{et al.}\textsuperscript{118}, and then implemented by Wang \textit{et al.}\textsuperscript{125}. Subsequently, this model was further adapted by Evans \textit{et al.} for the purposes of describing the transfer of labelled water from blood into the ventricular CSF, rather than into the cerebral cortex\textsuperscript{1,125,134} (Equation 4, Figure 7).
\( Ti \text{ range: } (Ti > \Delta t) \)

\[
\Delta M_{EV} = \frac{2M_0 f \alpha}{\varphi} \left\{ \exp(-Ti \cdot R_1) \cdot [\exp(\min(Ti, \Delta t + \tau) \cdot \Delta R) - \exp(\Delta t \cdot \Delta R)] \right. \\
\left. - \exp(\Delta t \cdot \Delta R)]/\Delta R \right\}
\]

\[ R_1 = \frac{1}{T_{1e}^b} \]

\[ \Delta R = \left( R_1 + \frac{f}{\varphi} \right) - \left( \frac{1}{T_1^b} \right) \]

**Equation 4 - Adapted 2-compartment Buxton kinetic model for quantification of BCSFB-mediated water delivery rates from BCSFB-ASL data (ultra-long TE, 220 ms).** Water delivery rates, \( f \) (red), are extracted within this thesis. \( \Delta t \) and \( \tau \) (blue) can either be fixed for higher precision extraction of \( f \), or extracted by modelling as an output parameter.

The general kinetic model defines a ‘tissue’ compartment within the imaging slice, that generalises the IV and EV contributions into a single compartment. In the general model, there are assumptions regarding the distribution of velocities and transit times in the tagged arteries which are required for the modelling. Alsop and Detre presented a theoretical framework and experimental data to more accurately account for transit effects in quantitative human perfusion imaging. They first derived the transit time sensitivity of the pASL expts, and then demonstrate that, by introducing a delay following labelling, transit effects are reduced, whilst maintaining sensitivity [see equation 5 from Alsop and Detre, 1996].

More specifically, their 2-compartment model not only redefines \( \Delta t \) to describe a transit time into the true tissue of interest, but also \( \delta_a \) – the transit time for the labelled bolus to reach the imaging slice. By having these separate arrival times, the proximal artery signal is now modelled and accounted for, and labelled spins may enter the tissues before imaging. Thus, this model (Figure 7) now directly accounts for the contribution of intraluminal/intravascular (IV) spins. The transit time of labelled blood water from the labelling slab to the vascular compartment of the imaging slice can be accounted for. This framework states that for single-TI PASL measurements, the TI value used must be large compared to the true tissue transit in
order to achieve insensitivity to transit time. This is satisfied in the rodent brain where $\Delta t$ is in the range of 100-300 ms, compared to TI values in the order of seconds (e.g. 2 s)\textsuperscript{134}.

These modifications of the model were primarily aimed at optimising CASL. Later, Wong \textit{et al.} proposed a single subtraction (QUIPSS II) method for PASL, which was based on the use of an inserted delay from the end of the inversion tag to the image acquisition ($w$)\textsuperscript{118}. The model now incorporates the times at which a saturation pulse is applied at a time, $T_{I1}$ (where $T_{I1} = \tau + w$), after the inversion pulse to externally define the tagging bolus duration. Images are then acquired at a later time, $T_{I2}$, where $T_{I2} = T_{I}$. For this saturation pulse-based method for PASL, the delay time ($\Delta T = T_{I2} - T_{I1}$) should be greater than $\delta_a$ in order to minimize the effects of transit time and intravascular tagged blood (see Wong \textit{et al.}, equations 2-5\textsuperscript{118}). This approach is further discussed and implemented in a study by Wang \textit{et al.}\textsuperscript{125}. However, BCSFB-ASL does not implement a saturation pulse, and so the introduced delay, $w$, is 0. As such, $T_{I2}$ is taken as the inflow time ($T_{I}$), and $T_{I1}$ is taken as $\tau$, as $T_{I2} = \tau + w$. Now, $\Delta T = (T_{I2} - T_{I1}) = (T_{I} - \tau)$.

The adapted 2-compartment model discussed thus far defines equations for magnetization at $T_{I} < \Delta t$ (prior to arrival into the true tissue) and $T_{I} > \Delta t$ (after arrival into the true tissue), which can now be used to better define compartments at the CP\textsuperscript{118,125,134}. The adaptation by Evans \textit{et al.} includes only the equation describing “extravascular signal”, $\Delta M_{EV}$, which applies to the measured BCSFB-ASL signal that derives from labelled water delivery to the CSF, i.e. the true “tissue” compartment. Here, an adjustment is made from the equations used in the work by Wang \textit{et al.}\textsuperscript{125}: the equation describing the signal contribution from intravascular spins, $\Delta M_{IV}$ (where $T_{I} < \Delta t$, before the arrival of the labelled bolus into ventricular CSF), is excluded. At $T_{I}$ values before $\Delta t$, the labelled water would not have crossed the BCSFB, and this signal would describe water still within the vasculature. Due to use of an ultra-long echo time (220 ms), signal arising from labelled water in blood are nulled\textsuperscript{122}. Overall, this adaptation allows for the model to be utilised to describe the delivery of labelled blood water into the CSF compartment, as opposed to brain tissue in standard-ASL (Figure 7, Equation 4).

As with the use of a general kinetic model for standard-ASL quantification, this adapted 2-compartment model requires several inputs: $\Delta M/M_{0\text{corr}}$, $T_{1CSF}$, inversion efficiency ($\alpha = 0.9$), and the longitudinal relaxation of arterial blood ($T_{1b} = 2.5$ s, from the literature\textsuperscript{135}). R1 is the
longitudinal relaxation rate of brain tissue in the absence of blood flow. Here, instead of using defining an equilibrium tissue/blood partition coefficient for water \( (\lambda = 0.9) \), we use a constant, \( \phi \), which defines the blood-to-CSF water partition coefficient\(^1\), which is now the density of water in the blood divided by the density of water in the CSF. As such, \( \phi \) is taken as 1\(^{1,124,136,137}\).

The model can be rearranged to extract water delivery rates from the \( \Delta M \) values obtained (\( f \)), as well as arrival time (\( \Delta t \)) and the temporal length of the tagged bolus (\( \tau \)). When collecting multi-TI BCSBF-ASL data within this thesis for kinetic modelling, there are instances where 3-parameter fittings are conducted to acquire values for all three parameters, e.g. for investigations into CSF spaces beyond the lateral ventricles (Chapter 4), or in pilot cohorts (Chapter 5). However, by using values of \( \Delta t \) and \( \tau \) from pilot cohorts and values from the initial characterisation of BCSFB-ASL in the mouse brain\(^1\), we can use fixed values of \( \Delta t \) and \( \tau \) to extract water delivery rates with a higher degree of precision.
a) $0 < TI < \Delta t$

Inversion plane

Proximal arteries

Labelled bolus of water

Vascular compartment

Tissue compartment

CSF

BCSFB

Imaging slice

$\delta_n = 0.2 \text{ s}$

$\Delta t = 0.61 \text{ s}$

b) $\delta_n < TI < \Delta t$

Inversion plane

Proximal arteries

IV water

Vascular compartment

Tissue compartment

CSF

BCSFB

Imaging slice

$\delta_n = 0.2 \text{ s}$

$\Delta t = 0.61 \text{ s}$

c) $\delta_n < TI < \tau + \Delta t$

Inversion plane

Proximal arteries

IV water

EV water

Vascular compartment

Tissue compartment

CSF

BCSFB

Imaging slice

$\delta_n = 0.2 \text{ s}$

$\Delta t = 0.61 \text{ s}$
**Figure 7 - 2-compartment Buxton model for BCSFB-ASL.** Separation of the tissue within the imaging slice (red) into: a vascular compartment with an “arterial transit” (δₐ), and a “true tissue” compartment with a “tissue transit time” (Δₜ), shown in blue (where the “tissue” is now the CSF compartment). Transit time values are taken from Evans et al. ¹. The general ‘tissue’ compartment from the single model (Figure xxx) is now divided into explicit intravascular (IV) and extravascular (EV) compartments. Δₜ and δₐ transit times now specifically define the time taken for the labelled bolus of water with temporal length (τ) to arrive at each compartment within the imaging slice¹¹⁸,¹²⁵,¹³⁴. BCSFB = blood-CSF barrier. Transit time values are taken from Evans et al. ¹. For clarity, the inversion plane shown here in green is more specifically used for CASL. For FAIR PASL, a much larger inversion plane is used, as depicted in Figure 3 (FAIR ASL labelling schemes). a) Prior to the arrival of the labelled bolus of water at the tissue (0 < TI < δₐ), the labelled bolus is present in the proximal vessels. b) When the labelled bolus is present within the tissue compartment (Δₜ < TI < τ + Δₜ), the labelled bolus of water has not yet crossed the BCSFB, but is instead present in the capillaries perfusing the BCSFB, and as such, can be considered IV water. c) When the entirety of the labelled bolus has entered the imaging slice (δₐ < TI < Δₜ + τ), there is subsequent delivery of labelled water across the BCSFB from the IV to ventricular CSF (extravascular, EV) compartment¹. Vessel component of diagram created with Biorender.com.

**Analytical pipeling for standard-ASL and BCSFB-ASL images** Having introduced the models utilised in this work for the quantification of absolute values of cerebral blood flow (CBF) and rates of BCSFB-mediated water delivery, I will now provide an overview of the analytical steps required to provide all inputs for the models described above (Figure 8 and Figure 9 below).
Figure 8 – Standard-ASL multi-TI analytical pipeline. Quantification of cortical CBF using data from MR images (standard-ASL multi-TI data (TE = 20ms)).
**Figure 9 – BCSFB-ASL multi-TI analytical pipeline.** Quantification of BCSFB-mediated water delivery using data from MR images (BCSFB-ASL multi-TI data (TE = 220 ms), T2-weighted anatomical reference data).

When analysing standard-ASL images, for each subject, a single ROI was drawn across the cortex of the brain using the slice-selective/labelled image, and the mean average signal was...
calculated across the voxels. This provided a single value for each slice-selective and non-selective image within a dataset. For each pair of images, each slice-selective image intensity (M_{s}) had its corresponding non-selective image intensity (M_{c}) subtracted to provide a ΔM value. Similarly, when analysing BCSFB-ASL images obtained at TE = 220 ms, a single ROI was drawn to include two regions of 2 × 3 voxels (12 voxels in total, Figure 10) that overlaid with the position of the lateral ventricles. When obtaining multiple repetitions at each TI, averaging of the M_{c} and ΔM signals was conducted prior to further model fittings.

![Figure 10 – ROI selection for BCSFB-ASL images. Example from representative C57/BL6 mouse shown on averaged control (non-selective FAIR) image (TI = 4 s, n = 1, 10 repetitions), alongside corresponding T2-weighted anatomical coronal reference.](image)

The Buxton kinetic modelling requires two further subject-wise inputs: M0 (equilibrium magnetisation) which will serve as a normalisation to ΔM (i.e. ΔM/M0), and longitudinal relaxation of the tissue of interest, either T1_{cortex} for traditional-ASL measures, or T1_{CSF} for BCSFB-ASL. M_{c} data across the array of Tis are used to fit a simple inversion recovery model (IR, Equation 5) to obtain these two values.
\[ M_C = M_0 \left( 1 - 2 \exp \left( - \frac{TI}{T1_{tissue}} \right) \right) \]

**Equation 5 - Simple inversion recovery (IR).** \( M_C \) data as a function of \( TI \) are used to fit this model in order to extract \( M_0 \) and \( T1 \) (either \( T1_{cortex} \) or \( T1_{CSF} \)).

For BCSFB-ASL quantification, subject-wise ventricular volume normalisation is required to provide volume-normalised, equilibrium magnetization (\( M0_{corr} \), Equation 6). This correction accounts for the total ventricular volume (quantified from T2-weighted anatomical images) and the ventricular ROI volume (i.e., from the 12 voxels used for the ROI from the low resolution functional data) \(^1\). By producing \( \Delta M/M0_{corr} \) values for the fitting procedure, this step is critical for the accurate quantification of the total amount of BCSFB-mediated water delivery; the calculated \( M_0 \) will be highly dependent on ventricle size due to partial volume effects in the low resolution ASL images \(^1\).

\[ M0_{corr} = M0 \times \frac{\text{Total ventricular volume}}{\text{Ventricular ROI volume}} \]

**Equation 6 – M0 volume correction for BCSFB-ASL data.** Provides a value of \( M0_{corr} \) by accounting for ventricular volume differences.

Having calculated subject-wise \( \Delta M/M0 \) (or \( \Delta M/M0_{corr} \)) and \( T1_{tissue} \) values, the data can now be used to fit to the appropriate Buxton kinetic model.
Single-TI measurements for dynamic time-course data

Thus far, the analytical pipeline described has focussed on the processing of data acquired at arrayed TI values. However, within this body of work, several studies are conducted using a fixed, single, TI value (single-TI), for example, during experiments which obtain a dynamic time-course to describe pharmacological modulation of CBF and BCSFB-mediated water delivery from a baseline in Chapter 2. In order to capture the changes in brain hemodynamics – BCSFB function and cortical CBF – we predominantly present the data within Chapter 2 as relative changes (i.e. $\Delta M/M_{\text{C}}$ normalised to the baseline). However, in Chapters 2 and 3 where single-TI measurements were conducted, Buxton kinetic modelling was conducted to obtain absolute rates of BCSFB-mediated water delivery or cortical CBF. Averaged $\Delta M/M_{\text{C}}$ values at a single TI are used as an input into the relevant Buxton kinetic model, and a single flow value is obtained. Thus, several further assumptions are made for values of $T_1_{\text{tissue}}$, bolus arrival times ($\Delta t$) and temporal length ($\tau$), all of which are obtained from previous measurements $^1$.

Furthermore, phMRI data acquired in Chapter 2 utilised a single-TI, interleaved-TE ASL approach to maximise sensitivity to, and maximise the temporal resolution to capture relative, rather than absolute, changes in two distinct hemodynamic parameters: cortical CBF and BCSFB function. We assume that changes in the ASL signal are reflective in changes of flow, as opposed to changes in bolus arrival time/arterial transit time ($\Delta t$) or length of the tagged bolus ($\tau$), neither of which are captured due to the single-TI nature of our measurements. We are also insensitive towards changes in arterial transit, due to the very short transit times observed in the mouse brain $^{138}$. As the animal remains in the same position within the volume coil, we reason that $\tau$ is very unlikely to markedly change due to the applied challenges. Together, therefore, it is reasonable to assert that changes in the $\Delta M$ signal (normalised to control) are proportional to changes in CBF or rates of BCSFB-mediated water delivery.

Imaging the rodent brain under anaesthesia

Rodent MRI studies are commonly performed under anaesthesia, as there are technical and ethical difficulties in implementing awake rodent imaging. Anaesthesia protocols are known to affect cerebral hemodynamics, in a manner that varies between strains of rodents $^{139,140}$. Thus, the anaesthetic regime, in conjunction with the particular rodent strain, will impact the
absolute values of CBF. As such, utilising a consistent/standardised protocol for each study is necessary to provide values of CBF, CVR, CSF dynamics, BCSFB-mediated water delivery etc., that are readily comparable between subjects. Isoflurane is a commonly used anaesthetic for MRI studies using mice, due to the straightforward route of administration (via a nosecone), fast reversibility and recovery of mice, the high scan-rescan reproducibility\textsuperscript{1}, and the minimal long-term effects within subjects \textsuperscript{1,141,142}. As a result of its vasodilatory effect, isoflurane has been shown to limit the sensitivity to any further vasodilatory agents when assessing CVR responses using ASL-MRI, for example, with a hypercapnia challenge \textsuperscript{142,143}. It is therefore necessary to also choose an anaesthetic regime which maximises sensitivity to the pharmacological intervention, which, in the case of a hypercapnia challenge, could be the vasoconstrictive agent, medetomidine \textsuperscript{142}.

Alterations in general and cerebral hemodynamics in rodents under anaesthesia can therefore limit the accuracy of quantification of hemodynamic parameters such as CBF, relative to the ground truth under normal/awake physiology. However, awake imaging is not only considered challenging due to technical and ethical limitations, but also due to concerns on the stress imposed on a mouse, and how this will subsequently impact measurements of hemodynamics and brain function \textsuperscript{144,145}.

On the other hand, it has been extensively argued that, in order for a seamless transition from pre-clinical research to clinical implementation to be possible, the use of awake imaging and identical imaging paradigms are a necessity \textsuperscript{146}.

Owing to the considerations described here, I have conducted imaging in the anaesthetised rodent brain within this body of work, primarily using isoflurane. As such, the hemodynamics investigated within this thesis, e.g. BCSFB function, CBF, CVR etc., should be taken in the context of the anaesthetised brain.

\textit{m. Thesis objectives}

This thesis is formed of several Chapters which aim to further optimise, characterise, and apply the BCSFB-ASL methodology with the purpose of non-invasively investigating \textit{in-vivo} function of the BCSFB system in both healthy and altered brain states.
Chapter 2 uses both traditional ASL and the recently-developed BCSFB-ASL MRI techniques towards the purpose of developing and applying a pharmacological MRI (phMRI) cerebrovascular reactivity protocol for the unique, non-invasive measurement of BBB and BCSFB ‘reactivity’ – i.e. their blood flow responses to a pharmacological or gas ‘challenge’. These results will provide novel insights into challenge-induced alterations to choroid plexus blood flow in response to three challenges — hypercapnia 25–27, caffeine 23,147, and vasopressin 28,29— in the anaesthetized adult mouse brain. Furthermore, by studying an aged mouse model, we aim to investigate age-related dysfunctionality of the BCSFB, which may manifest as differential reactivity towards a vasopressin challenge, relative to their adult counterparts 55–58.

The initial development and validation of the BCSFB-ASL methodology by Evans et al., as well as the phMRI experiments described in Chapter 2, were conducted on the Agilent 9.4T MRI system. Chapter 3 describes the steps taken to characterise and optimise the BCSFB-ASL methodology on the Bruker 9.4T system, and thereby utilise the enhanced hardware and software features to enhance the BCSFB-ASL imaging technique. By doing so, we seek to improve the precision of the measurement, as well as facilitating a broader range of applications for the methodology for future studies with a focus on the CP-CSF-BCSFB system (given the much wider availability of Bruker pre-clinical MRI scanners compared to Agilent).

The application of BCSFB-ASL has been centred on the study of the caudal aspect of the lateral ventricles, which is rich in choroid plexus tissue 1,106. Recent literature has revealed the presence of ultra-long TE ASL signals in the human brain not only at the choroid plexus, but has also shown to be distributed in the subarachnoid spaces 132. Applying the optimised BCSFB-ASL methodology on the Bruker system, the experiments conducted in Chapter 4 aim to characterise the kinetics of the BCSFB-ASL signal in regions beyond that of the caudal lateral ventricles, i.e. in CSF regions which have either a high or low choroid plexus tissue density. Through this investigation, we hope to further understand other sources of the ultra-long TE ASL signal, and how it may be influenced by phenomena such as rapid CSF mixing and non-BCSFB mediated routes of labelled blood water delivery.

Lastly, Chapter 5 provides further application of BCSFB-ASL to investigate the effects of systemic hypertension on BCSFB function in a Spontaneous Hypertensive Rat (SHR) model. Recent studies suggest that the BCSFB displays a selective vulnerability to hypertensive
damage that may initiate downstream neurodegenerative processes. However, these studies used invasive, ex-vivo methodologies to detect functional irregularities at the BCSFB locus of SHRs. Taking advantage of the non-invasive nature of BCSFB-ASL, we obtain data to non-invasively quantify the differences in BCSFB-mediated water delivery rates, which could serve as a potential sensitive early biomarker for hypertensive-driven neurodegeneration, whilst also providing mechanistic insights. We also investigated cortical CBF changes – a conventional biomarker of cerebrovascular health. In addition, we aim to assess differences in brain fluid management through alterations in $T_{1\,CSF}$ and ventricular volumes.
2. Chapter 2 – Pharmacological MRI with Simultaneous Measurement of Cerebral Perfusion and Blood-Cerebrospinal Fluid Barrier Function using Interleaved Echo-Time Arterial Spin Labelling

a. Introduction

The blood-brain-barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) mediate the complex interplay between blood and the brain, which is a core homeostatic mechanism supporting healthy brain function. The main locus of the BCSFB is the choroid plexus, which resides in the brain’s fluid filled ventricles. We have recently developed an MRI technique for the non-invasive assessment of BCSFB function, by quantifying the rate of BCSFB-mediated delivery of endogenous arterial blood water to ventricular cerebrospinal fluid (CSF) \(^1\). As such, this translational approach (termed blood-cerebrospinal fluid barrier arterial spin labelling [BCSFB-ASL]) may be useful to better understand the precise role of the BCSFB in conditions such as Alzheimer’s disease and multiple sclerosis, where dysfunction has been postulated to be mechanistically significant \(^47,148\). Importantly, this technique allows repeated measures with high temporal resolution, which non-invasively enables dynamic capture of the BCSFB’s functional response to drugs, challenges, and disease.

Estimates of cerebral blood flow (CBF) provide a surrogate measure of the functionality of the BBB to, for example, facilitate a constant supply of oxygen and nutrients from the blood to the highly metabolically active brain cells. CBF can be measured non-invasively with standard arterial spin labelling (ASL) MRI\(^149\). ASL also provides repeated measures and so can be used to measure dynamic changes in CBF in response to a drug (an approach which falls under a set of methods known as pharmacological MRI [phMRI] \(^150,151\)). phMRI provides a non-invasive means of assessing the spatial-temporal dynamics of new and emerging drugs to alter brain function and/or the brain’s vascular properties. Such measurements can also provide novel
diagnostic and/or mechanistic insight into brain pathology by examining how underlying
disease states modulate the brain’s vascular response to a drug \(^{99-101}\). As described in Chapter
1 (Literature Review), Evans \textit{et al.} utilised a phMRI approach to capture changes in BCSFB
function using a vasopressin challenge, towards the purpose of validating the BCSFB-ASL
methodology \(^{1}\). Distinctly, Evans \textit{et al.} employed an approach that provided measures of
BCSFB function and cortical-CBF before and after challenge administration. The single time-
point measurements did not capture the simultaneous, dynamic time course of the
vasopressin-induced changes in BCSFB-ASL function and cortical CBF simultaneously \(^{1}\).

Due to the relatively long half-life of most drugs compared to the duration of an MRI scan, it
is typically only possible to measure the functional response to a single dose in a given phMRI
session. This limits the scope of MRI scan-types applied to characterize pharmacological
perturbation of brain hemodynamics in real time. Indeed, to date, typically either ASL or T2*
weighted blood-oxygenation-level-dependent (BOLD) measurements have been recorded in
phMRI studies.

Here, we exploit the overlap of the traditional ASL and BCSFB-ASL MRI techniques and employ
an interleaved echo time (TE) ASL sequence to capture the simultaneous response of two
distinct components of brain physiology to a single dose of a drug or ‘challenge’: i) parenchymal tissue perfusion, and ii) rates of BCSFB-mediated arterial blood water delivery
to the CSF (a surrogate, non-invasive, measure of BCSFB function). This approach provides an
efficient means to better understand the differential response of vessels that comprise the
BBB and BCSFB to pharmacological perturbation in the healthy and diseased brain. We first
apply this method to reproduce the well-established specific vasoconstriction of vessels that
perfuse the choroid plexus (with no decrease in parenchymal perfusion) that is induced by
antidiuretic hormone, vasopressin\(^{1,28}\). Then, we investigated whether the commonly used
vasodilatory agent, \textit{CO}\(_2\), is a viable approach to dilate the vasculature of the BCSFB. Finally,
given experimental data linking caffeine consumption to marked changes in choroid plexus
physiology, we proceeded to measure the effect of caffeine on non-invasive measures of
BCSFB function in the mouse brain.

There is wide-ranging evidence that marked deterioration of BCSFB structure and function
occurs in the aged brain, with increased levels of endogenous vasopressin hypothesized to be an
important mechanism underlying this decline \(^{53,55-58}\). Therefore, to investigate the
mechanistic link between BCSFB function and vasopressin in the aged brain, using non-invasive measurements, we applied the interleaved-TE ASL technique to capture the phMRI response to vasopressin in a cohort of aged and adult mice. Together, our findings represent the first phMRI measurements of BCSFB function using interleaved-TE ASL, and based on the marked differential response to vasopressin in the aged brain, highlight the potential of this approach to better understand the mechanisms that underlie age related cognitive decline.

The work presented in this chapter is an adaptation of published research (see Inclusion of Published Work (1)), and as such, covers work not included in the final publication. For example, I first describe the optimization of several experimental parameters used for vasoreactivity phMRI experiments using hypercapnia specifically, such as the choice of anaesthetic regime and experimental timings. By investigating these experimental choices and determining the optimal conditions, I then proceeded to implement interleaved-TE ASL phMRI using a hypercapnia challenge in a larger cohort of adult mice. In addition, I apply an interleaved-TE ASL approach to investigate the effect of caffeine and vasopressin in adult mice. I then extended the use of this ASL platform to conduct a study comparing the reactivity to a vasopressin pharmacological challenge in adult vs aged mice, to determine whether there is an age-related impairment in reactivity at the BCSFB. Post-hoc, exploratory analysis is also presented in this chapter which aims to investigate the effects of anaesthetic on baseline BCSFB-mediated water delivery rates.

b. Methods

Data collection

All data within this chapter were collected solely by myself (see Inclusion of Published Work (1)).

Animal preparation

All animal procedures were performed under the UK Home Office Act (Scientific Procedures, 1986). C57/BL6 female WT mice (provided by Charles River Laboratories) were used for the pharmacological experiments conducted only in adult mice, as well as the pilot studies to optimise the hypercapnia protocol. When investigating the effects of ageing on the brain, 14 aged mice (C57BL/6JRj 23-months old, male) and 14 strain-matched adult-mice (5-months
old, male) were used, provided by Janvier Labs (France). Further details of sample sizes, dosing and administration routes for the pharmacological/gas challenge experiments are outlined in Table 1. For each experimental stage, e.g. pilot study, interleaved-TE ASL phMRI implementation in solely adult mice, and for the ageing study, experimental aims and hypotheses are detailed in Table 2.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>n</th>
<th>Pharmacological/Gas challenge</th>
<th>Dose</th>
<th>Administration route</th>
<th>Anaesthetic</th>
<th>Baseline (mins)</th>
<th>Challenge (mins)</th>
<th>Recovery (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 Adult</td>
<td>2</td>
<td>CO₂ (pilot)</td>
<td>10%</td>
<td>Nose cone inhalation</td>
<td>ISO</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C57BL/6 Adult</td>
<td>3</td>
<td>CO₂ (pilot)</td>
<td>10%</td>
<td>Nose cone inhalation</td>
<td>MED</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>C57BL/6 Adult</td>
<td>9</td>
<td>Saline (vehicle)</td>
<td>5 ml/kg</td>
<td>Intraperitoneal bolus</td>
<td>ISO</td>
<td>10</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6 Adult</td>
<td>6</td>
<td>Vasopressin</td>
<td>47 U/kg</td>
<td>Intraperitoneal bolus</td>
<td>ISO</td>
<td>10</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6 Adult</td>
<td>6</td>
<td>Caffeine</td>
<td>50 mg/kg</td>
<td>Intraperitoneal bolus</td>
<td>ISO</td>
<td>10</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6 Adult</td>
<td>8</td>
<td>CO₂</td>
<td>10%</td>
<td>Nose cone inhalation</td>
<td>MED</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>C57BL/6JRj Adult (5-months)</td>
<td>14</td>
<td>Vasopressin</td>
<td>47 U/kg</td>
<td>Intraperitoneal bolus</td>
<td>ISO</td>
<td>10</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6JRj Aged (23-months)</td>
<td>14</td>
<td>Vasopressin</td>
<td>47 U/kg</td>
<td>Intraperitoneal bolus</td>
<td>ISO</td>
<td>10</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1 – Pharmacological/gas challenge administration.** Details of the mouse strain and sample sizes used for our pharmacological/gas challenge experiments, alongside the doses and administration routes for each of the selected challenges, as well as the anaesthetic regimes. Timings of the experimental phases have been shown: baseline, challenge (“on” time), and recovery (for the hypercapnia challenge only). ISO = Isoflurane anaesthetic regime, MED = Medetomidine anaesthetic regime.

Prior to commencing MRI acquisitions, subjects underwent anaesthetic induction using 2% isoflurane in 0.8L/min medical air and 0.2L/min O₂. Following induction and weighing, mice
were placed into the MRI cradle with bite bar, nose cone and ear bars to ensure a well secured position of the mouse head to minimise motion during the data acquisition. Eye ointment was also applied to prevent drying.

For subjects receiving vasopressin, caffeine, or saline vehicles, an intraperitoneal infusion line was attached to each subject to allow for the delivery of drug solution boluses whilst the animal was positioned within the magnet bore. The length of the line allowed manual injection from outside the scanner bore, without needing to reposition the cradle. Therefore, data collection was not interrupted whilst administering the challenge, and could continue immediately after challenge administration. After a 10-minute baseline, the drug solution was injected using this infusion line and measurements were obtained for a further 20 minutes (Table 1).

For subjects receiving a hypercapnia challenge, data collection was again uninterrupted, as a CO$_2$ cylinder was connected to the medical air delivery unit outside the magnet to enable the switching of gases within this experiment, i.e. without having to remove subjects from the cradle. Experimental timings and anaesthetic regimes were varied from pilot experimentation to the final implementation (Table 1), which is further described below.

A scavenger pump was placed inside the magnet bore to prevent isoflurane build-up. Anaesthesia was maintained during the acquisition by reducing isoflurane concentration to 1.5% in 0.4L/min medical air and 0.1L/min O$_2$.

For all subjects, temperature and breathing rate were monitored throughout all the experiments using a rectal probe and a respiration pad (SA Instruments). Mouse temperature was maintained at 37 ± 0.5°C using a combination of heated water tubing and warm air flow during both the induction and data acquisition stages. Breathing rate was maintained at 120 ± 10 breaths/minute.

**Pilot study – optimising hypercapnia vascular reactivity protocol**

Preliminary experiments were conducted towards the aim of developing a reliable methodological protocol to capture an increase in cortical CBF to a hypercapnia challenge in
the anaesthetised mouse brain, using a traditional-ASL protocol (non-interleaved, described below). These experiments utilised only C57BL/6 adult mice (Table 1, Table 2).

**Isoflurane regime (ISO):**

For pilot CO₂ experiments conducted under isoflurane using shorter phases (5 mins per phase, Table 1, n = 2), a low isoflurane anaesthetic regime was used (ISO): induction using 2% isoflurane in 0.8L/min medical air and 0.2L/min O₂, and maintenance with 1.5% isoflurane in 0.4L/min medical air and 0.1L/min O₂.

**Medetomidine regime (MED):**

Subsequent experiments in the pilot cohort (n = 3) and the larger cohort used in our final study (n = 8) were conducted with longer experimental timings (10 mins per phase, Table 1) and utilised a medetomidine anaesthetic regime (MED). Anaesthetic induction and initial maintenance, prior to MRI data acquisition, were conducted using isoflurane (as described above). Once in the cradle, a separate infusion line was attached subcutaneously to allow for the delivery of a 0.4 mg/kg bolus of medetomidine (5% in saline) via a Syringe Pump (Infuse/Withdraw PHD 22/2000, Harvard Apparatus). Following this, isoflurane concentration was reduced to 0.5% with 1.0L/min medical air, well before commencing dynamic MRI data capture. During the acquisition, anaesthesia was additionally maintained by subcutaneously infusing the medetomidine at a rate of 0.8 mg/kg/hour.

**Hypercapnia administration:**

Pilot hypercapnia experiments conducted using the ISO regime (n = 2) were conducted with a 5-minute baseline (medical air), followed by a 5-minute CO₂ “on” period (10%, 1L/min in medical air), and a 5-minute CO₂ “off” recovery period (medical air) (Table 1).

For the hypercapnia protocol in mice undergoing the MED regime during the pilot studies, as well as the final cohort, after obtaining a 10-minute baseline, CO₂ (10%) was delivered at 1L/min in medical air for 10 minutes (n = 3 pilot cohort, n = 8 final cohort). Subsequently, CO₂ was replaced again by medical air for another 10 minutes to induce a recovery period (Table 1).
Intraperitoneal pharmacological challenge administration:

For the administration of intraperitoneally delivered challenges (saline, caffeine, vasopressin), a 10-minute baseline was acquired prior to injection. Within the same scan, and in an uninterrupted manner, an IP dose of the challenge was given, and interleaved-TE ASL MRI measurements continued for a further 20 minutes (Table 1). These experimental choices were used for studies conducted in adult C57BL/6 mice for the implantation of interleaved-TE ASL phMRI for the exploration of changes in vessel tone with selected IP drug challenges, as well as for the ageing study conducted with vasopressin in C57BL/6JRj mice (Table 1, Table 2).
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pharmacological / gas challenge</th>
<th>Aim/Hypothesis</th>
<th>Subjects</th>
<th>Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilot – optimisation of hypercapnia protocol Standard-ASL</td>
<td>CO₂</td>
<td>Optimisation of the hypercapnia protocol anaesthetic and experimental timings to sensitively capture CO₂-induced increases in cortical CBF, as measured with standard-ASL (non-interleaved).</td>
<td>Adult mice (C57BL/6)</td>
<td>Individual subject responses: Internal control – challenge phase relative to pre-challenge baseline. Statistical significance of pharmacologically-induced effect within group determined by paired t-test of baseline to mean signal of challenge phase.</td>
</tr>
<tr>
<td>phMRI implementation – adult mice Interleaved-TE ASL</td>
<td>Saline</td>
<td>Vehicle control – controlling for the effects induced by the intraperitoneal injection of a fluid (i.e. the vehicle for delivery of caffeine and vasopressin drug challenges)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vasopressin</td>
<td>Hypothesis – vasopressin will induce a specific, exclusive decrease in BCSFB-water delivery, with minimal change in cortical CBF.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>Investigate the effects of caffeine on BCSFB function – given that caffeine is a potent modulator of BCSFB physiology — and investigate cortical CBF.</td>
<td>Adult mice (C57BL/6)</td>
<td>Individual subject responses: Internal control – final 10-minute challenge phase relative to pre-challenge 10-minute baseline. Statistical significance of pharmacologically-induced effect within group determined by paired t-test of baseline to mean signal of challenge phase (final 10 minutes).</td>
</tr>
<tr>
<td></td>
<td>CO₂</td>
<td>Investigate possible CO₂-driven changes to vessel tone in the choroid plexus, as well as reproducing established vasodilation in cortex with an optimised protocol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phMRI implementation – ageing study Interleaved-TE ASL</td>
<td>Vasopressin</td>
<td>Investigate age-related impairment in BCSFB reactivity to a vasopressin pharmacological challenge using a non-invasive interleaved-TE ASL.</td>
<td>Strain matched C57BL/6JRj mice: Adult (5-months) vs aged mice (23-months)</td>
<td>Individual subject responses to vasopressin: Internal control – relative to pre-challenge baseline. Comparison of reactivity: comparison of BCSFB reactivity and cortical CBF response in adult (control) vs aged mice</td>
</tr>
</tbody>
</table>

**Table 2 – Experimental stages and aims.** Outline of the various experimental stages within this chapter, alongside the pharmacological/gas challenges used, the aim/hypothesis of each stage, and the comparisons made.
**Magnetic resonance imaging (MRI) protocols**

Images were acquired using an Agilent 9.4T imaging system with a 72mm volume coil for RF transmission and a two-channel array surface coil receiver (Rapid Biomedical), positioned on top of the head.

**Figure 1 – Example MR images:** a) Sagittal anatomical reference image with overlaid FAIR-ASL imaging slice plan (single slice, 2.4mm). b) Subtracted (global – slice selective) interleaved-TE ASL image obtained at TE = 20 ms. c) Subtracted (global – slice selective) interleaved-TE ASL image obtained at TE = 220 ms with overlaid lateral ventricular ROI used during analysis. D) Coronal anatomical reference images displaying imaging volume across the ventricles (6x 0.4mm slices, 2.4mm total).

**Anatomical reference scans**

Anatomical reference structural images were acquired with T2 weighting in order to clearly visualise the location of the major CSF compartments in the mouse brain using a fast-spin echo sequence (FOV = 25mm × 25mm; matrix size = 256 × 256; echo train length = 8; Teeff = 48ms; TR = 5s).
Sagittal anatomical reference images (8 slices, 1mm slice thickness) were used to position the axial anatomical reference imaging slice and the ASL imaging slices (Figure 1a).

Coronal anatomical reference images (6x 0.4mm slices, 2.4mm total) were manually positioned to align with the caudal region of the lateral ventricles to display the corresponding imaging volume across the ventricles (Figure 1d).

**FAIR-ASL scans**

Each of the ASL imaging protocols was based on the FAIR sequence \(^{152}\) with a single shot SE-EPI readout, slice-selective width = 19.2mm, and a global labelling pulse, across all the experiments.

Parameters for standard-ASL and BCSFB-ASL: single slice, 2.4mm slice thickness, TR = 12000 ms, TI = 4000 ms, matrix size: 32 x 32, FOV: 20mm x 20mm, inversion pulse BW = 20,000 Hz, shape = sech. 38 repetitions were used to cover a 30-minute scan protocol. TE = 20 ms for standard-ASL, and TE = 220ms for BCSFB-ASL.

Importantly, the ASL imaging slice was manually positioned to align with the caudal end of the lateral ventricles, as it has been previously shown to be the predominant region within the lateral ventricles which the CP occupies \(^{106}\) (Figure 1a). Therefore, as described previously measurements of BCSFB function are concentrated to CP within the later ventricles and not the 3\(^{rd}\) and 4\(^{th}\) ventricle \(^{1}\).

In order to interleave a traditional-ASL measurement with the BCSFB-ASL measurement, an additional array loop was added to switch between two echo time values: 220 ms (BCSFB-ASL) and 20 ms (traditional-ASL). As shown in Figure 2 in a single repetition of the interleaved-ASL technique (48 seconds), there are 4 ASL images obtained, each with a TR of 12 s: standard-ASL control image (20 ms), standard-ASL labelled image (20 ms), BCSFB-ASL control image (220 ms), BCSFB-ASL labelled image (220 ms). This loop continues for a given number of repetitions. Representative interleaved-TE ASL images are displayed in Figure 1b (TE = 20 ms) and Figure 1c (TE = 220 ms).
Interleaved-TE ASL MRI was used for all acquisitions after the initial pilot study (n = 2, 3 hypercapnia optimisation), which utilised standard-ASL imaging (TE = 20 ms only). Interleaved-TE ASL was used in all phMRI experiments in adult C57BL/6 mice when investigating the effects of the three challenges (vasopressin, caffeine, CO₂), as well as in the ageing study using a vasopressin challenge in adult and aged C57BL/6j mice. Although the BCSFB-ASL signal (TE = 220 ms) and the cortical CBF data (TE = 20 ms) were acquired simultaneously, I opted to present the dynamic time courses for each of these distinct measures separately, for the purposes of visual clarity.

**Image processing and analysis for relative blood flow quantification**

When analysing standard-ASL images obtained with TE = 20 ms, a single ROI was drawn for each subject across the cortex of the brain using the slice-selective FAIR image, and the mean voxel signal was calculated across the ROI. For each ASL image pair, the non-selective mean ROI value was subtracted from the slice-selective mean ROI value to provide the perfusion-weighted signal ΔM, which was then divided by the corresponding control magnetisation (non-selective signal, M₀).

For the BCSFB-ASL images obtained with TE = 220 ms, two 3x2 voxel ROIs (12 voxels in total) were positioned on a slice-selective image, overlaid with the position of the lateral ventricles.
(Figure 1c). As with the standard-ASL analysis, the combined ROI average signals were subtracted in a pairwise fashion to provide $\Delta M$ values.

For both standard- and BCSFB-ASL data, in a subject-wise manner, $\Delta M/M_c$ values were divided by the mean $\Delta M/M_c$ value for the 10-minute baseline to provide a measure of relative, baseline-normalised blood flow. The average normalised ASL signal during baseline was taken and compared to the average normalised signal following the administration of vasopressin/caffeine or during the period of $CO_2$ administration, using a paired t-test. A ten-minute analytical window at the end of the challenge period was used for IP challenges (vasopressin, caffeine, saline). The full ten-minute window was also averaged for the hypercapnia challenge period. Kinetic modelling was not used at this particular stage, and data is presented as relative changes of the BCSFB-ASL or standard-ASL signal. At this stage of technique validation, this summary measure provides a straightforward and conservative quantification of the degree of response towards pharmacological or gas challenges.

**Mono-exponential model of ASL response to pharmacological challenges**

The mean time-course data for vasopressin and caffeine were used to fit a simple mono-exponential decay model for the purpose of providing further visualisation of responses towards pharmacological challenges. A piecewise function was used, with the following equation:

$$y = \begin{cases} 
  b, & x < 10 \\
  b \cdot \exp(d \cdot (x - 10)), & x \geq 10 
\end{cases}$$

where $x = \text{time (mins)}$, $y = \text{relative blood flow}$, $b = \text{baseline blood flow}$, $d = \text{mono-exponential decay constant}$. The switching point, $x = 10$ mins, corresponds to the point when challenges were administered following the baseline.

**Control conditions**

To control for the effects induced by the intraperitoneal injection of a fluid, e.g. for the case of vasopressin or caffeine administration, we administered a saline control (5 ml/kg).
Statistical analysis

Statistical comparisons between baseline (first 10 minutes) and challenge (last 10 minutes) experimental phases were conducted using paired, 2-tailed, Student’s t-tests (Microsoft Excel). When comparing the cortical-CBF and BCSFB-ASL reactivities to a vasopressin challenge between adult and aged mice, unpaired, 2-tailed, Student’s t-tests were used (Microsoft Excel).

Post-hoc analysis: absolute quantification of ASL-MRI data

For the post-hoc, exploratory analysis of anaesthetic effects, absolute cortical CBF values and absolute BCSFB-mediated water delivery values were quantified using the general Buxton kinetic model ($\Delta M/M_0$ values at $TE = 20$ ms) and the adapted 2-compartment Buxton kinetic model ($\Delta M/M_{0,corr}$ values at $TE = 220$ ms) previously described in Chapter 1 (Literature Review - Section I)\textsuperscript{1,118,125,134}.

c. Results

Interleaved-TE ASL measurements were obtained to investigate the effects of vasopressin, CO$_2$, and caffeine on cortical perfusion and rates of BCSFB-mediated arterial blood water delivery to ventricular CSF in the C57BL/6 WT adult mouse brain (n = 6, Table 1, Table 2). Administration of saline vehicle to account for the delivery of fluid intraperitoneally, revealed very subtle changes in cortical perfusion, and no measurable change in the BCSFB-ASL signal (ASL signal: 2.3% mean change from baseline, $p = 0.03$; BCSFB-ASL signal: -0.9% mean change from baseline, $p = 0.92$; n = 9, Figure 3g, h, Figure 4a,b).
Figure 3 – Interleaved echo time ASL: cortical BF and BCSFB-ASL simultaneous responses to selected pharmacological/gas challenges. Top row: vasopressin. Second row: caffeine. Third row: CO₂ (following a distinct experimental routine compared to the IP challenges). Averaged time courses alongside the mono-exponential model fittings are shown for cortical BF (a, c, e,) and the BCSFB-ASL signal (b, d, f). Bottom row: bar plots displaying group-averaged relative changes from baseline during a window in the challenge period, for cortical BF (g) and the BCSFB-ASL signal (h). Error bars: ±SEM.
**Figure 4 — Interleaved echo time ASL: cortical BF and BCSFB-ASL simultaneous responses to selected challenges.** Individual subject data showing baseline vs challenge comparison for relative cortical BF changes (left column: a, c, e, g) and BCSFB-ASL signal changes (right column: b, d, f, h). Top row: saline vehicles (n = 9), second row: vasopressin (n = 6), third row: caffeine (n = 6), bottom row: CO\(_2\) (n = 8).

**Vasopressin**

Evidence from previous invasive measures, as well as previous non-interleaved, BCSFB-ASL acquisitions in our lab, has highlighted the ability of vasopressin to cause specific vasoconstriction at the BCSFB (with no decrease in cortical perfusion) \(^{1,28}\). Figure 3 (a, b) shows the simultaneous response of cortical perfusion and the BCSFB-ASL signal to a vasopressin challenge in 6 mice, using the interleaved echo-time ASL phMRI protocol. Averaged dynamic cortical blood flow time course data revealed a significant, 15% average increase from baseline upon vasopressin administration (p = 0.010, Figure 3g). Conversely, the BCSFB-ASL time course data revealed a significant vasopressin-induced decrease (mean change 58%, p = 0.00004, Figure 3h). Individual subject responses to a vasopressin challenge are shown in Figure 4 (c, d). In summary, as hypothesised, the application of a pharmacological vasopressin challenge evoked significant and specific downregulation of BCSFB function from baseline values, with no evidence for a decrease in cortical perfusion.

**Caffeine**

Caffeine is a safe and commonly consumed drug \(^{153}\) which has been repeatedly implicated as a significant modulator of BCSFB physiology \(^{154}\). Here we explored the potential of caffeine to pharmacologically challenge the BCSFB, with functional changes captured using non-invasive methods for the first time. Figure 2 (c, d) shows the simultaneous response of cortical blood flow and rates of BCSFB-mediated blood water delivery to the CSF in response to a caffeine challenge. Imposing a caffeine challenge revealed significant decreases in the BCSFB-ASL signal by an average of 46% (p = 0.0002, Figure 2d, h). However, caffeine administration did not evoke significant changes in cortical perfusion (p = 0.27, Figure 2g). Individual subject responses to caffeine administration are displayed in Figure 4 (e, f).
**Pilot study – optimising hypercapnia vascular reactivity protocol**

CO\(_2\) is a vasodilatory agent commonly used to challenge the brain’s vasculature, with the extent of the haemodynamic ‘reactivity’ often interpreted as a measure of cerebrovascular health\(^\text{99–101,150}\). Here we were interested in investigating possible CO\(_2\)-driven changes to vessel tone in the choroid plexus using MRI for the first time.

Preliminary experiments were conducted to optimise the methodological protocol to capture the well described hypercapnia-induced increases in cortical CBF\(^\text{99–101,150}\). Here, we applied a non-interleaved, traditional-ASL protocol (Figure 2). The optimised experimental timings and anaesthetic regime would then be applied to investigate possible CO\(_2\)-driven changes to cortical CBF and BCSFB function, simultaneously, using non-invasive techniques for the first time.

Using an isoflurane anaesthetic regime and 5 minutes per phase, the averaged dynamic functional data (Figure 5a) shows no clear increase in cortical CBF following challenge administration. This is reiterated when comparing the mean ASL signals at baseline vs challenge with individual subject data: non-significant, 1.9% increase (p = 0.29, Figure 5c).

Upon switching to the medetomidine-based anaesthetic regime and 10 minutes per phase (Table 1), a greater increase in CBF was observed in the averaged dynamic time course data (Figure 5b). We detected a significant, average increase in CBF of 22% during the challenge phase (p = 0.009, Figure 5d).

**Hypercapnia (CO\(_2\)) – final implementation**

Given the increased sensitivity to detecting a hypercapnia-induced elevation in cortical perfusion from baseline values, we applied the optimised hypercapnia protocol in a larger cohort (n = 8) to capture the simultaneous effect of CO\(_2\) on cortical CBF and BCSFB function with interleaved-TE ASL (Figure 3e,f, Figure 4 g,h). As expected, following the administration of a hypercapnia challenge, there was a significant increase in cortical perfusion (p = 0.0038), averaging 21% (Figure 2g). Furthermore, significant increases in the BCSFB-ASL signal from baseline were also detected upon inducing hypercapnia averaging 21% (p = 0.031, Figure 2h).
Figure 5 — Hypercapnia challenge protocol optimisation. Top row: using an isoflurane anaesthetic regime and 5 minutes per phase. Bottom row: using a medetomidine regime and 10-minutes per phase. A) and c) Interleaved dynamic time course data for relative CBF changes using a 10% hypercapnia challenge. B) and d) Individual animal, relative CBF responses to a hypercapnia challenge.

BCSFB Reactivity to Exogenous Vasopressin is Attenuated in the Aged Brain

Previous studies have reported elevated levels of endogenous vasopressin with ageing.\textsuperscript{52–54} As vasopressin is associated with reduced CP perfusion (see, for example, Figure 3d), this is thought to contribute to the established impairment of CP structure and function in the aged brain.\textsuperscript{58}
To further probe the mechanistic interaction between BCSFB function, vasopressin and brain ageing using non-invasive methods, we applied the interleaved-TE ASL technique to a cohort of adult (5-months, n = 14) and aged (23-months, n = 14) mice to measure the response of the vessels within the BCSFB to a vasopressin challenge. We hypothesised a reduced response to exogenous vasopressin in the aged cohort, owing to the established elevation of endogenous levels of vasopressin in blood plasma with ageing 53.

Figure 6 and Figure 7 show the cortical BF and BCSFB-ASL signal responses following vasopressin administration, in 14 adult and 14 aged mice, using an interleaved-TE ASL approach. We observe significant increases in cortical blood flow following vasopressin administration in adult mice averaging 19% (p = 0.003, data Figure 6a, b, Figure 7a, b). Increases in average cortical perfusion in aged mice bordered significance (11% average p = 0.057, Figure 6a, b, Figure 7a, b). Post-hoc analysis revealed that there was no significant difference between the cortical reactivity to a vasopressin challenge between the adult and aged cohort (p = 0.28).

As expected, vasopressin induced a marked decrease in the BCSFB-ASL signal in the adult cohort, averaging 40% (p = 0.00002, Figure 6c, d, Figure 7c, d). In contrast, the response to vasopressin in the aged cohort was dampened, averaging a 6.8% decrease (not significant, p = 0.64, Figure 6c, d, Figure 7c, d). The marked 6-fold impairment in the response of the BCSFB-ASL signal observed in aged mice vs their adult counterparts was statistically significant (p = 0.046, Figure 6d).
**Figure 6 – Interleaved echo time ASL: group-averaged adult vs aged response to vasopressin.** Top row: cortical BF response to vasopressin. Bottom row: BCSFB-ASL signal response to vasopressin. Averaged time courses alongside the mono-exponential model fittings are shown for both adult and aged cohorts, for cortical-BF (a) and the BCSFB-ASL signal (c). Bar plots display averaged relative changes in the adult and aged groups relative to baseline, for cortical BF (b) and the BCSFB-ASL signal (d). Error bars: ±SEM.
Figure 7 - Interleaved echo time ASL: individual subject responses response to vasopressin in adult vs aged mice. Ageing study individual subject data showing baseline vs challenge comparison for relative cortical BF changes (top row: a, b) and BCSFB-ASL signal changes (bottom row: c, d). Group-averaged baseline vs challenge values are shown for cortical BF (red line) and BCSFB-ASL signal (blue line).

**Anaesthetic effects**

As described in Chapter 1 (Literature Review – Section I), anaesthetics have been shown to affect rodent physiology and more specifically, hemodynamics in the brain. Previous studies have assessed the effect of different anaesthetic regimes on CBF measurements and the reactivity to a CO₂ challenge with ASL; the application of a medetomidine anaesthetic regime was shown to lower baseline cortical CBF. Using an ultra-long TE, recent work by Lee et al. showed that dexmedetomidine, the active enantiomer of medetomidine, results in a significant, 14% reduction of BCSFB water flow in rats. In our work, exploratory analysis
was conducted to investigate how anaesthetic choice affected quantified values of absolute, baseline cortical perfusion, as well as rates of BCSFB-mediated labelled water delivery into ventricular CSF. We compared isoflurane baseline data (ISO, saline vehicle dataset, n = 9), to medetomidine baseline data (MED, hypercapnia experiments, n = 8) (Table 1, Figure 8).

A general Buxton kinetic model was fit to ΔM/M₀ data to extract absolute CBF (Figure 8a below, and Chapter 1 - Section I). In line with the literature, we found that there was a significant, 31% decrease in baseline CBF when switching from the isoflurane to medetomidine regime ([ISO = 243 ml/min/100g], [MED = 168 ml/min/100g], p = 8 × 10⁻⁵, unpaired 2-tailed t-test, Figure 8a).

For BCSFB-ASL, a corrected M₀ (M₀corr) values were calculated to account for individual subject ventricular volumes, as described in Chapter 1. BCSFB-ASL ΔM/M₀corr values from BCSFB-ASL measurements were then used to fit an adaptation of the 2-compartment Buxton kinetic model, providing quantified rates of BCSFB-mediated water delivery (Figure 8b below, and Chapter 1, Section I). Similar to the work by Lee et al. using dexmedetomidine, a marked, 74% decreases in baseline rates of BCSFB-mediated water delivery were observed under medetomidine, relative to isoflurane ([ISO = 21.8 ml/min/100ml], [MED = 5.7 ml/min/100ml], p = 6 × 10⁻⁶, unpaired 2-tailed t-test, Figure 8b).

![Graphs showing comparison of cortical BF and water delivery between ISO and MED](image-url)
**Figure 8 – Anaesthetic regime effects on baseline CBF and BCSFB-mediated water delivery values.** Absolute perfusion/water delivery values quantified using Buxton kinetic modelling: a) cortical BF, b) BCSFB-mediated water delivery rates. Error bars: ±SEM. N = 9 (ISO), n = 8 (MED).

**d. Discussion**

To date, studies investigating BCSFB function *in-vivo* have been limited in number. Historically, obtaining measurements of choroid plexus perfusion necessitated the use of highly invasive techniques with injectable radiotracers or contrast agents alongside terminal surgical procedures, and hence were limited in their temporal resolution and translatability \(^{155,156}\). The BCSFB-ASL technique provides a surrogate measure of BCSFB function by quantifying the rate of BCSF—mediated delivery of endogenous arterial blood water to ventricular CSF, by using an ultra-long TE (220 ms @ 9.4T), low spatial resolution readout ASL sequence. Here, by arraying the ultra-long and short TEs used for BCSFB- and traditional-ASL, respectively, and keeping all other parameters constant, the interleaved-TE ASL approach provides a platform to non-invasively monitor simultaneous, dynamic changes in brain parenchymal perfusion and BCSFB-function in the mouse brain.

Here, we demonstrate the application of this sequence to the field of phMRI, where it may be particularly advantageous, since typically only a single dose of drug can be administered in a single imaging session. We demonstrate differential responses to vasopressin and caffeine, i.e., an increase or no change in cortical CBF vs a marked decrease in BCSFB function, highlighting the distinct physiology of the BBB associated with vessels that perfuse the cortex and the BCSFB within the CP. Moreover, we apply the method to probe the mechanistic interaction between BCSFB function, ageing, and vasopressin, recording a dampened pharmacological response at the BCSFB to exogenous vasopressin in the aged mouse brain. As such, for the first time, this work describes an impairment in BCSFB function within the aged mouse brain, measured non-invasively with ASL-MRI.

The BCSFB-ASL signal reflects the average rate of perfusion to the CP convolved with the permeability of the BCSFB to water (i.e. the ‘extraction fraction’ \(^{157}\)) and the mass of the CP tissue (in this case, within the lateral ventricles). It is important to note, therefore, that this measurement reflects the rate of delivery of labelled blood water across the BCSFB rather
than the net secretion of CSF. We speculate that changes to the BCSFB-ASL signal driven by pharmacological/gas challenges measured here are primarily driven by alteration in vessel tone at the BCSFB, in turn modulating CP perfusion. For example, vasopressin is known to cause vasoconstriction at the CP but increase vessel permeability to water; we measure a decreased BCSFB-ASL signal, suggesting that vasoconstriction is the dominant mechanism. Indeed, vasopressin evoked a marked (58%) decrease in BCSFB-mediated blood water delivery to the CSF. Reproducing this finding, previously reported both with an invasive microsphere approach and with un-interleaved BCSFB-ASL measurements, demonstrates the sensitivity of interleaved-TE ASL to detect an exclusive downregulation in BCSFB function, as hypothesised.

Hypercapnia-induced cerebrovascular reactivity (CVR) measurements using MRI-based approaches are frequently conducted both pre-clinically and clinically. Dampened dilatory responses to CO₂ have been implicated in many pathological conditions affecting the brain microvasculature, such as cognitive decline in ageing and dementia, hypertension, as well as being associated with a higher risk of ischemic injuries. We report significant increases in cortical perfusion under hypercapnia in the mouse brain, as hypothesised, alongside simultaneous increases in the BCSFB-ASL signal to a similar magnitude. Despite the established increases in CBF following CO₂ administration, there is limited and conflicting literature detailing the effects of hypercapnia to CP perfusion: hypercapnia has been shown to significantly increase CP blood flow in sheep by 27%, with other studies contradicting this finding by reporting an approximately 2-fold decrease in response to CO₂. Autoradiography measurements in rats did not capture any CO₂-driven changes in CP blood flow. Our results indicate a significant, CO₂-driven increase in CP perfusion of a similar magnitude to that observed in the cortex.

Measurements of caffeine-induced CBF changes in humans and rat models have shown global and regional CBF decreases. However, there are currently no available reports detailing the effects of caffeine on CBF in the mouse brain specifically. We provide dynamic data covering the immediate effects of caffeine, with no significant decreases in cortical BF observed here. The lack of significant decrease in cortical BF observed here may reflect a type II error, as prior studies would suggest any putative decrease to be relatively subtle. Nonetheless, our data clearly demonstrates the magnitude of caffeine-driven decreases
in the BCSBF-ASL signal to be markedly greater than changes in cortical perfusion, shown to be significant through post-hoc testing, (p = 0.0006). Caffeine has been found to be a potent modulator of BCSFB physiology \(^{154}\). Indeed, the marked decrease of the BCSFB-ASL signal suggests that the vasculature of the BCSFB is more dramatically affected by caffeine-induced vasoconstriction than the vessels in the cortex. The dose applied here, when allometrically scaled for human administration, equates to approximately 2 espresso coffee shots, thus keeping translatability as an important consideration \(^{164}\). By virtue of the convenient and commonplace ingestion of caffeine outside the realms of blood flow studies in humans, caffeine becomes an ideal candidate for studying the functional response of the BCSFB in a future clinical setting.

Ageing, although not a disease, is the primary risk factor for neurodegenerative disease. The study of an aged model is useful for the discovery of these early biomarkers which, if left unchecked, may seed downstream neurodegeneration. There is increasing evidence pointing to morphological changes and functional impairment in the BCSFB which occur with physiological ageing and age-associate diseases, as reported in several histological, in-vitro, and in-vivo studies \(^{1,41,46,48,49,92,165}\). It has been proposed that a major player in the onset of the age-related derangement of BCSFB function is the formation of dark CPeCs, driven by increases in endogenous plasma vasopressin levels \(^{52-54,165}\). We hypothesised that as a result of the functionality and structural changes at the BCSFB, combined with the increased level of vasopressin within the aged brain, the aged mouse brain will display a decreased magnitude of reactivity towards a vasopressin challenge relative to their strain-matched adult counterparts. Aged mice were shown to have a significant, dramatic reduction, in their reactivity to a vasopressin challenge when compared to adult mice. As such, this finding represents the first demonstration of a differential response of the BCSFB to a pharmacological challenge across different brain states (in this case the aged vs adult mouse brain). It would be interesting to apply the methods to mouse models of neurodegenerative conditions such as AD in future studies.

In this work, blood pressure was not recorded. However, this is unlikely to be a significant confound for data interpretation as: i) in the case of caffeine, several studies have shown that at clinically relevant doses, caffeine causes a relatively subtle increase in BP that is well within
autoregulatory limits \(^{166}\); ii) for \(\text{CO}_2\), previous studies have shown that increased CBF is primarily driven by local vasodilation in the brain and not systemic changes in BP \(^{167,168}\); iii) vasopressin is known to increase BP, however we observe a significant decrease in the BCSFB-ASL signal, suggesting that local changes in vessel tone are the dominant mechanism underlying this change. In addition, changes in blood pressure will not impact the modelling of the ASL signal in this study, as described in Chapter 1 (Section e, Section I).

Currently, the intraperitoneal dose of vasopressin used in our experiments equates to approximately 45 U/kg. This dose, was chosen to provide a robust and reproducible response to maximise sensitivity in the mechanistic study on the ageing mice, based on previous measurement in our lab\(^1\). Allometric conversion to a human dose gives an approximate dose of 4 U/kg. Vasopressin is used clinically, albeit not for vascular reactivity protocols, at a dosing range 1-2 orders of magnitude lower than our chosen dose \(^{169,170}\). Thus, there is potential for refinement in the dosing, delivery, and/or imaging paradigm to enable the measurement of BCSFB functionality without requiring such large doses. Promisingly, recent work has provided encouragement that it is possible to measure apparent choroid plexus perfusion using ASL techniques in the human brain \(^{171,172}\). Therefore, it may also be possible to apply these methods clinically, to investigate relative changes in CP perfusion in response to a drug or challenge, as performed here using the BCSFB-ASL approach.

Given that anaesthesia has been shown to impact rodent physiology, and specifically cerebral hemodynamics \(^{139,140}\) (Chapter 1 – Section I), it was necessary to choose an anaesthetic regime which would afford an increased sensitivity to hypercapnia-driven vasodilation at the cortex – a hemodynamic response which has been well characterised \(^{98-101}\). Isoflurane has an established dose-dependent vasodilatory effect, which results in an increased baseline CBF \(^{142,143}\). Medetomidine, on the other hand, is known to be a vasoconstrictive agent, and as such, will reduce baseline CBF as well as being shown to provide higher CVR values \(^{142}\). These differences in baseline CBF are also reproduced in this work (Figure 5). Importantly, Munting \textit{et al.} displayed that hypercapnia-induced CVR was negatively correlated with baseline CBF, due to the competing vasodilatory effects of \(\text{CO}_2\) and isoflurane. In order to maximise CVR responses and improve sensitivity towards detecting an established vasodilatory effect with hypercapnia, pilot experiments were conducted to decide between the use of an isoflurane vs medetomidine protocol. Our initial experiments reiterated that the use of a medetomidine-
based protocol would enable higher CVR values under hypercapnia, owing to the lower baseline CBF values under medetomidine.\footnote{142}

Furthermore, exploratory analysis was conducted to elucidate the effects of anaesthetic regime choice, i.e. medetomidine or isoflurane, on baseline cortical CBF, and more importantly, novel insights into baseline BCSFB-mediated water delivery rates. Recent literature utilising CASL-MRI at an ultra-long TE has revealed that dexmedetomidine, the active enantiomer of medetomidine, induces a 14\% reduction in BCSFB water flow in the rat brain.\footnote{124} We found that medetomidine induced a 31\% reduction in cortical perfusion, compared to a 74\% reduction in rates of BCSFB-mediated water delivery. This analysis provided a strong indication that medetomidine’s vasoconstrictive effects apply not only to vessels supplying parenchymal tissue, but also extend to vessels perfusing the choroid plexus. Given the difference in vasoconstrictive potency compared to vessels perfusing the cortex, the vasculature supplying the choroid plexus may have a greater sensitivity to the use of medetomidine. Anaesthetic regime choice is a critical consideration when conducting studies related to glymphatic clearance in the brain, as alterations in perfusion of the choroid plexus and BCSFB function influence rates of CSF-mediated solute transport pathways.\footnote{124,173} It is important to consider the technical challenges to clinical translation of the method. As shown in our recent work, the BCSFB signal across the lateral ventricles is markedly smaller than the standard ASL signal from the parenchymal tissue (primarily reflecting the relatively small volume of CP tissue within the lateral ventricles and the long TE).\footnote{1} Nonetheless, we are able to detect relatively small signals from labelled blood water that has been delivered to the CSF compartment during the TI, in comparison to the signal detected from our TE = 20 ms (standard-ASL) measurement $\Delta M/M_0 = 0.034 \pm 0.003$ TE = 20 ms, vs 0.004 ($\pm 0.001$) at TE = 220 ms (saline group average, $n = 9$). This corresponds to an estimated cortical perfusion of 243 ($\pm 8$) ml/100g/min and rate of BCSFB-mediated labelled blood water delivery to the CSF of 21.8 ($\pm 2.6$) ml/100ml/min.

In the human brain, reliable measurement of the BCSFB signal will be made challenging by the lower flow rates and increased arterial transit times relative to the mouse brain as well as the decreased T1 of the blood water at clinical field strengths. This will be offset, however, by the large increase in brain volume and CP tissue as well as modern sequence and hardware innovations such as multi-channel receiver coils. Encouragingly, measurements of the
exchange time ("tissue transit time-arterial transit time) of labelled water across the BBB suggests similar timescales between the mouse and human\textsuperscript{122,174}, suggesting that the transfer of labelled water across the BCSBF should occur within clinically relevant ASL timing parameters (labelling duration and PLD) in the human brain.

An interesting feature of the measurement is that a low-resolution readout can be used to boost sensitivity due to lack of partial volume effects from blood and tissue at ultra-long TE. As such, the BCSBF-ASL technique aims to capture a measure of the total amount of BCSFB-mediated labelled blood water delivery to the lateral ventricles as a surrogate index of BCSFB function within the lateral ventricles. In this way the integral of the BCSFB signal is taken across the entire lateral ventricles meaning that this measure will be independent of ventricle volume and location of the CP within the lateral ventricles. Importantly, the low-resolution imaging comes at little cost since, unlike imaging of the blood brain barrier, where parenchymal vascular delivery often has high spatial affinity to the location of tissue metabolism, the measures of BCSFB function have little need for high spatial resolution because the material delivered from the blood to the CSF is immediately dispersed around the ventricular compartment due to CSF pulsation.

A limitation of this study is the lack of a control group that was scanned for the 30-minute protocol without undergoing any intervention. We have used a saline vehicle control to account for the injection of fluid intraperitoneally, which revealed that the BCSFB-ASL signal was not impacted, and that there were significant, yet subtle changes to cortical CBF. This saline control group has not been designed to account for scanner drift. Drift in the measured signal can severely compromise data quality. However, it is important to note that the use of an ASL MRI acquisition is advantageous in this case for a phMRI protocol due to the calculation of a subtracted, $\Delta M$ signal. As the control ($M_C$) and labelled ($M_L$) images are acquired in an interleaved fashion themselves, along with the arrayed echo times which are also interleaved in this particular implementation, this mitigates the effects of scanner drift due to the normalisation by the control signal, $M_C$. In contrast, a gradient-echo EPI BOLD-based functional MRI would be more susceptible to scanner drift effects\textsuperscript{175,176}.

There are difficulties in implementing an interleaved protocol. Firstly, there is a reduction in temporal resolution for each of the two individual aspects of cerebral hemodynamics we are investigating. In other words, purely quantifying the BCSFB-ASL signal would increase the
effective temporal resolution of measuring BCSFB-mediated water delivery and its pharmacological modulation (2 measurements within 48 s). By interleaving, we are also able to capture cortical CBF, which lowers the temporal resolution of both measurements (1 measurement of each within 48 s). Considering the implementation of this methodology on other MR systems, such as the Bruker 9.4T, it may be required to utilise pulse programming due to the non-conventional nature of this sequence.

In conclusion, our results illustrate the value of an interleaved-TE ASL MRI approach to quantify pharmacologically-induced changes to vessels that make up the BBB in the cortex and the BCSFB in the choroid plexus. Caffeine appears to be a promising candidate to challenge the vasculature of the BCSFB, owing to the marked response of the BCSFB-ASL measurement to this safe and readily available drug. In response to vasopressin, an aged cohort displayed a marked impairment in BCSFB ‘reactivity’, relative to an adult cohort. We also demonstrate the marked vasoconstrictive effects of a medetomidine anaesthetic regime on the choroid plexus, relative to isoflurane anaesthesia. Importantly, these results highlight the capability of such measurements to be utilised as a biomarker for probing altered functionality and pathophysiology in the aged or diseased brain, providing a potential novel biomarker of age-related cognitive decline.
3. Chapter 3 – Optimising BCSFB-ASL on the Bruker 9.4T System

a. Introduction

The Agilent 9.4T imaging system used to obtain the data described within this thesis thus far is now over a decade old and, like many pre-clinical Agilent MRI scanners across the world, it may soon be decommissioned due to the decision by Agilent Technologies to discontinue this arm of their company. Our laboratory also has a new Bruker 9.4T Biospec scanner installed, which provides the opportunity to improve the BCSFB-ASL imaging technique by taking advantage of the enhanced MRI hardware and software features. This will not only contribute towards an improvement in the signal-to-noise ratio (SNR), i.e. the accuracy and precision, of the BCSFB-ASL measurement (and by extension, the interleaved-ASL measurement), but will hopefully also permit a wider scope of applications for the technique, as the methods developed here can subsequently be used by Bruker users with an interest in studying the choroid plexus-BCSFB system.

The Bruker imaging system has several enhanced features that could benefit the implementation of the BCSFB-ASL technique for the assessment of BCSFB-mediated water delivery into ventricular CSF. The Bruker gradient set (BioSpec B-GA 12S2) has a 10% higher gradient amplitude (440 mT/m) compared to the 9.4T Agilent system (400 mT/m) which can increase the imaging speed. With matching echo planar imaging (EPI) sequence imaging parameters, the ramp time for the EPI readout is also markedly shorter on the Bruker (50 μs) compared to the Agilent system (120 μs) which could reduce susceptibility of the sequence to motion. The improved hardware enables shorter EPI inter-echo spacing, thereby reducing distortions in the phase-encoding direction making higher resolutions (beyond 32x32 matrix sizes) more feasible.

There are several key differences in the surface coils for the imaging systems that are used in conjunction with the volume coil for reception and transmission of the signal, respectively. Importantly, compared to the 2-channel mouse brain surface coil used with the Agilent system, the Bruker system utilises a circularly polarized shaped, 4-channel surface coil for
reception of the signal. The circularly polarized design has optimal sensitivity for the mouse head and the increased number of channels will improve the signal-to-noise ratio (SNR)\textsuperscript{121}.

EPI is very sensitive to static magnetic field (B0) inhomogeneities that produce nonlinear geometric distortion primarily along the phase-encoding direction. These artifacts become more severe at higher magnetic field strengths, e.g. 9.4T\textsuperscript{177}. Using Paravision v6.0.1 (Bruker software) for the Bruker imaging system, a FieldMap-based shimming approach (MAPSHIM) can be used to optimize B0 field homogeneity. The FieldMap provides quantitative maps of the static magnetic field B0 scaled in resonance frequency units. Following the acquisition of a 3D double gradient echo dataset, the reconstruction performs a phase difference calculation, a noise-robust phase unwrapping and a conversion to a frequency map. This procedure can be automatically performed in a user-selected selected region of interest, enabling the experimenter to achieve an increased homogeneity compared to the Agilent manual global shimming.

In this chapter, we sought to first re-implement the BCSFB-ASL MRI approach on the Bruker 9.4T system. Subsequently, by taking advantage of the aforementioned hardware and software improvements of the Bruker system, we also aim to optimise several features of the acquisition: SNR, temporal resolution, and labelling efficiency, which, when taken together, provide an increased confidence in the accuracy and precision of the measurement. To achieve this, the default Perfusion\textsubscript{FAIR\_EPI} sequence available on the Paravision v6.0.1 system was taken as the starting point, which would normally be used for traditional FAIR-ASL measurements of cerebral perfusion.

Several experiments were conducted to characterise the measurement, similar to the validation steps used in the initial development of the BCSFB-ASL method\textsuperscript{1}. Here, I present the various steps explicitly to provide clarity over the optimisation timeline, particularly as the outcome of each experiment and dataset directly informed on the next experiment conducted with the latest iteration of the Perfusion\textsubscript{FAIR\_EPI} sequence.
**b. Methods**

**Data collection**

All the data collected towards the aims in this chapter were collected by me.

**Animal preparation and anaesthetic induction/maintenance**

All animal procedures were performed under the UK Home Office Act (Scientific Procedures, 1986). C57/BL6 WT mice (provided by Charles River Laboratories) were used for the experiments during the methodological development process.

Animal preparation, anaesthetic induction and maintenance under isoflurane, and the physical set-up of subjects in the mouse imaging cradle (Classic MRI Animal Cradle, Bruker) were conducted using an identical approach to the studies outlined in Chapter 2 - Methods Development.

**Magnetic resonance imaging (MRI) protocols**

Images were acquired on a 9.4T Bruker imaging system (BioSpec 94/20 USR) with a horizontal bore and 440 mT/m gradient set with outer/inner diameter 205 mm /116 mm respectively (BioSpec B-GA 12S2), 86 mm volume coil and a circularly polarized four-channel array mouse brain surface coil for the transmission and the reception of the radiofrequency (RF) signal, respectively. The position of the mouse brain was placed symmetrically in the iso-centre to maximise the quality of the shim.

**Anatomical reference scans**

A T2-TurboRARE sequence (Paravision v6.0.1) was used to collect anatomical reference structural images to clearly visualise the location of the major CSF compartments in the mouse brain, using a fast-spin echo, T2-weighted readout (FOV = 20 mm × 20 mm; matrix size = 256x256; RARE factor = 8; TEeff = 33 ms; TR = 2500 ms).

Using the same slice geometry as the experiments conducted on the Agilent 9.4T system (as described in Chapter 2), sagittal (8 × 1 mm slices) and coronal (6 × 0.4 mm slices) anatomical reference images were collected. The coronal images were used for the positioning of the ASL imaging slices at the caudal end of the lateral ventricles, and for the calculation of lateral ventricular volume by manual segmentation.
**FAIR-ASL scans using single-TI and multi-TI approaches**

The base Perfusion_FAIR_EPI imaging sequence offered by Paravision v6.0.1 (Bruker software) uses flow-alternating inversion recovery (FAIR) with a spin-echo (SE), single-shot echo planar imaging (EPI) readout. This default Perfusion_FAIR_EPI sequence was subject to several points of alteration throughout the course of the optimisation experiments conducted within this chapter, for the purpose of more accurately and precisely capturing standard-ASL and BCSFB-ASL measurements.

Field inhomogeneities were improved through pre-scan automatic shim adjustments. Prior to running the Perfusion_FAIR_EPI sequence (and any optimised standard-ASL or BCSFB-ASL variants), Paravision v6.0.1 (Bruker software) was used for FieldMap-based shimming (MAPSHIM) to optimize B0 field homogeneity over the imaging field of view (FOV).

Parameters for standard-ASL and BCSFB-ASL: single slice, 2.4 mm slice thickness, matrix size: \(32 \times 32\), FOV: \(20 \text{ mm} \times 20 \text{ mm}\), dummy scans ([standard-ASL = 4], [BCSFB-ASL = 6]). TE = 20 ms for standard-ASL, and TE = 220 ms for BCSFB-ASL. Inversion pulse shape, bandwidth and non-selective thickness were optimised throughout the course of the experiments, which were finally selected to be the “Calculated” shape option provided by Paravision v6.0.1, with a bandwidth of 20 kHz, and a thickness of 20 mm.

**Single-TI ASL**

When collecting dynamic time course data (e.g. a baseline with 20 repetitions), a single-inflow time (TI) approach was used. For standard-ASL measurements, TI = 2000 ms, TR = 5000 ms. For BCSFB-ASL measurements, TI = 4000 ms, TR = 12000 ms. To improve our temporal resolution, several adjustments were made to further reduce the BCSFB-ASL repetition (TR) time/recovery time (TRec, Figure 1) in later experiments.
**Figure 1 – Constant Recovery Time (TRec)** – a) Sequence diagram of the FAIR-ASL MRI method, depicting the difference between having a constant repetition time (TR), vs a constant...

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recovery time. \( TI = \) inflow time, \( TE = \) echo time, \( RF = \) radiofrequency pulse. b) Table displaying how the effective recovery time at a given \( TI \) value varies when using \( TRec \) or \( TR \) settings.

**Multi-TI ASL**

When collecting multi-inflow time (TI) datasets, the desired TI values were arrayed within a single Perfusion\_FAIR\_EPI scan. Standard-ASL TI value array = \([200, 500, 1000, 1500, 2000, 3000, 4000, 6000 \text{ ms}]\), using TR = 12000 ms. BCSFB-ASL TI value array = \([200, 750, 1500, 2750, 4000, 5000, 6000 \text{ ms}]\). Initial experiments used a constant repetition time (TR) setting, which was then optimised across the study when validating our measurements, as described in the Results section, i.e. switching to a constant recovery setting (\( TRec \)).

**Image processing and analysis for blood flow quantification**

In order to obtain absolute, quantified values of cortical blood flow and BCSFB-mediated water delivery from dynamic time course data collected at a single inflow time (single-TI, \( TI = 4000 \text{ ms} \)), as well as multiple, arrayed inflow times (multi-TI), an analytical pipeline was used as described in Chapter 1 – Literature Review (see Chapter 1 - Section I Figure 8 and Figure 9). This pipeline, and any further adaptations for this chapter, has been summarised below.

When analysing standard-ASL images obtained with \( TE = 20 \text{ ms} \), a single region of interest (ROI) was drawn for each subject across the cortex of the brain using a non-selective (control) FAIR image, and the mean voxel signal was calculated across the ROI. For each ASL image pair, the non-selective mean ROI value (\( M_c \)) was subtracted from the slice-selective (labelled) mean ROI value to provide the perfusion-weighted signal \( \Delta M \).

For the BCSFB-ASL technique, our approach was to take the sum of the BCSFB - ASL signal in the lateral ventricles (see Chapter 1 - Section I, Figure 10). This would enable the measurement of the total amount of labelled arterial-blood-water delivery to ventricular CSF, thereby yielding an overall measure of total BSCFB function in the lateral ventricles. Therefore, for the BCSFB-ASL images, two \( 2 \times 3 \) voxel ROIs (12 voxels in total, ROI volume = \( 11.25 \text{ mm}^3 \)) were positioned on a slice-selective image, overlaid with the position of the lateral ventricles. As with the standard-ASL analysis, the combined ROI average signals were subtracted in a pairwise fashion to provide \( \Delta M \) values, with an added step of subject-wise ventricular volume normalisation to provide volume-normalised, equilibrium magnetization
This correction accounts for the total ventricular volume (quantified from T2-weighted anatomical images) and the ventricular ROI volume (i.e. from the 12 voxels used for the ROI from the low resolution functional data) \(^1\). This step is critical for the accurate quantification of the total amount of BCSFB-mediated water delivery; the calculated M\(_0\) will be highly dependent on ventricle size due to partial volume effects in the low resolution ASL images \(^1\).

Repeated measures of \(\Delta M\) and \(M_c\) values were averaged for each TI providing [TI, \(\Delta M\)] and [TI, \(M_c\)] datasets. The [TI, \(M_c\)] data were used for fitting a simple inversion recovery curve, permitting the extraction of T1 and M0 for each subject. The T1 of the CSF and of cortical brain tissue was calculated from the control images acquired at TE = 220 and 20 ms respectively. The [TI, \(\Delta M\)] values were then fit to the relevant Buxton model. The subject-wise T1 (T1\(_{\text{CSF}}\) for BCSFB-ASL or T1\(_{\text{cortex}}\) for standard ASL) and M0 (or M0\(_{\text{corr}}\) for BCSFB-ASL) values extracted from the IR fittings were used in the Buxton model as inputs when calculating CBF and BCSFB-mediated water delivery values for each subject \(^1\). For the quantification of cortical CBF, a single-compartment Buxton kinetic model approach was used \(^{133}\), with BCSFB-ASL requiring a 2-compartment adaptation of this model \(^{1,125,134}\). The latter adaptation aims to more accurately account for transit effects and intraluminal spins, ultimately allowing for the model to be utilised to describe the delivery of labelled blood water into the CSF (compartment), as opposed to extra-vascular brain tissue in standard-ASL.

The outputs of the model fittings provided subject-wise quantitative values for cortical perfusion (standard-ASL images at TE = 20 ms), and rates of BCSFB-mediated water delivery (BCSFB-ASL images at TE = 220 ms). We report the mean average of the output BCSFB-mediated water delivery rate, cortical CBF and T1\(_{\text{CSF}}\) across all subjects per group.

For the purpose of visual comparison of the group-averaged kinetic curves, for example, to highlight the impact of an optimised feature on the ability to obtain a kinetic curve with a good fit, we averaged the values of \(\Delta M/M0\) for standard-ASL, or \(\Delta M/M0_{\text{corr}}\) for BCSFB-ASL.

**Adapted methods**

For the purposes of optimisation and characterisation of the BCSFB-ASL methodology on the Bruker 9.4T system, various parameters were altered. For example, slice selective inversion
slab thickness, inversion pulse shape and bandwidth (BW), repetition time (TR)/recovery time (TRec), and imaging slice positioning.

**Statistical methods**

Statistical significance to determine the success of various optimisation procedures was conducted using paired, 2-tailed, Student’s t-tests (Microsoft Excel). The strength of the linear relationship between two variables, e.g. distance from the caudal LV and rates of BCSFB-mediated water delivery, was assessed using Pearson’s correlation coefficient (Microsoft Excel).

c. **Results and Discussion**

**Inversion slab thickness**

The default Perfusion_FAIR_EPI sequence utilises a slice-selective (label) inversion slab thickness that matches the width of the imaging slice thickness, which in this case was 2.4 mm. Using a water-filled phantom (n = 1), this thickness was gradually increased from 2.4 mm to 20 mm, in order to identify the thickness at which zero ASL signal ($\Delta M/M_0$) from the phantom could be measured (Figure 2). At each of the slab thickness values (2.4, 5.0, 10, 15, and 20 mm), a 20-repetition baseline was obtained at a single-TI, using the following MRI parameters: TI = 2000 ms, TR = 5000 ms, TE = 20 ms.

Figure 2 highlights that increasing the inversion slab thickness from the 2.4 mm default value resulted in a decrease in the measured ASL signal, which subsequently reaches a plateau at 15 mm. A finalised value of 20 mm was chosen, as this provided a desired $\Delta M/M_0$ value of 0 (i.e. no ASL signal when there is no flow). The 20 mm thickness is also in line with the 19.2 mm inversion thickness used on the Agilent 9.4T system, which was chosen to ensure that all CSF spaces within the brain are covered by the slice selective inversion. By doing so, CSF is inverted identically in the global and slice selective acquisitions, thereby preventing the effects of CSF motion confounding our measurement.
Figure 2 - Optimising inversion slab thickness – water phantom. Thickness values: [2.4, 5.0, 10, 15, 20 mm]. Error bars: ± stdev (20 repetitions).

Partial Fourier Transform

To optimise the BCSFB-ASL variation of the Perfusion_FAIR_EPI sequence further, the Partial Fourier Transform (Partial-FT) acceleration parameter was changed. Setting the phase encoding partial FT acceleration to a number higher than 1.0 reduces the matrix size by the inverse of the value of the acceleration factor, and shifts the position of the central echo towards the beginning of the echo train. The default perfusion EPI sequence has partial-FT switched “on”, at a value of ‘1.5’ in the phase-encoding direction, i.e. meaning that 1/1.5 (~67%) of k-space was captured in the phase encoding direction. We switched from the phase-encoding partial-FT ‘1.5’ “on” setting, to a value of ‘1.0’ “off”, thereby capturing the entirety of k-space. Baseline BCSFB-ASL measurements consisting of 20 repetitions at a single-TI were obtained in 3 mice (TI = 4000 ms, TR = 12000 ms, TE = 220 ms). For both modes of acquisition, frequency-encoded partial-FT was not active (fixed value of ‘1.0’, “off”)

A comparison of the standard deviation of the ASL signal across repeated measurement before and after turning partial-FT off (Figure 3a) revealed that the standard deviation value across the 3 mice decreased from an average of 14.9 ml/min/100ml, to 3.9 ml/min/100ml. This 4-fold improvement in the standard deviation was shown to be significant when using paired 2-tailed t-tests (p = 0.016, n =3). Quantifying absolute values of BCSFB-mediated water
delivery yielded a mean value of 7.1 ml/min/100ml when partial-FT was switched on, compared to 8.8 ml/min/100ml when turned off (Figure 3b). Changes in mean rates were found to not be significantly different (p = 0.23)

Figure 3 - Partial-FT effect on BCSFB-ASL data. Comparison of BCSFB-ASL measurement standard deviation (a) and mean rates of water delivery (b) from 20 repetitions taken at a single-TI (TI = 4 s) using BCFSB-ASL (n = 3). Partial-FT: ['on': (1.5,1.0)] vs ['off': (1.0,1.0)]

Partial-Fourier MRI acquisitions reduce the 2D MR imaging time by taking advantage of the conjugate symmetry of k-space e.g. in the case of the standard-ASL measurement at TE = 20 ms. This approach can also be advantageous for the purposes of reducing the EPI train length to reduce the echo time and thus increase image SNR. However, in the case of the BCSFB-ASL measurement using an ultra-long, 220 ms TE, partial-FT imaging would not be required. Furthermore, using partial-Fourier imaging methods, less data is acquired and therefore SNR is inherently reduced. It may be the case that when we use a TE of 220 ms, the asymmetrical sampling of k-space using partial-FT, there are phase errors in k-space which prevent an accurate estimation of data with conjugate symmetry. We speculate that these phase errors are impacted by the lower SNR of the BCSFB-ASL signal, compared to a traditional-ASL signal at a lower TE. Therefore, by collecting information from the entirety of k-space, we have demonstrated a markedly more stable measurement.
Multi-TI Buxton kinetic modelling – initial data

Following the investigation into inversion slab thickness and partial-FT, $\Delta M/M_0$ data as a function of TI, i.e. multi-inflow time (multi-TI) ASL data was obtained in a similar manner to our group’s previous work\(^1\). Fitting this Buxton kinetic model (and the appropriate adaptations) to the multi-TI data would determine whether the standard-ASL and BCSFB-ASL permit the extraction of physiologically meaningful perfusion/water delivery values with higher confidence\(^{1,125,133,134}\). Multi-TI data were separately collected for both standard-ASL and BCSFB-ASL in 3 subjects ($n = 3$, Figure 4). Using the updated Perfusion_FAIR_EPI sequence, the TE and TR parameters were changed to measure either cortical CBF with standard-ASL (TE = 20 ms, TR = 12000 ms) or BCSFB-mediated water delivery with BCSFB-ASL (TE = 220 ms, TR = 12000 ms), with multiple TI values arrayed (20 repetitions at each TI, see methods section).

**Figure 4 - Buxton kinetic modelling of multi-TI ASL data – initial data.** Group-averaged multi-TI data from standard-ASL (a) and BCSFB-ASL (b) measurements, used for fitting the general kinetic model and 2-compartment model, respectively. Error bars: ±SEM ($n = 3$).

As with previous standard- and BCSFB-ASL Buxton modelling data from our group ([Chapter 1 - Section g, Figure 5] and [Figure 1b from Evans et al., 2020\(^1\)]), we observe two kinetic curves...
which have distinct shapes reflecting the higher arrival time of the labelled bolus (Δt) and lower rate of T1 decay of labelled arterial water crossing the BCSFB into ventricular CSF.

Curve Fitting Toolbox (Matlab) was used within this chapter (and throughout this thesis) to fit kinetic curves to the acquired multi-T1 standard-ASL and BCSFB-ASL data to the models described in Chapter 1 (Literature Review – Section I). Fitting the Buxton kinetic model to the group-averaged ΔM/M0 data from standard-ASL measurements provided a **cortical CBF value of 170 ml/min/100g**. Multi-T1 BCSFB-ASL averaged ΔM/M0corr data were used to fit the 2-compartment adaptation of the kinetic curve and yielded a **water delivery rate of 14.5 ml/min/100ml**. Previous baseline measurements obtained at a single-T1 on the Agilent system indicated a cortical CBF of 243 ± 8 ml/100g/min, and a BCSFB-mediated water delivery rate of 21.8 ± 2.6 ml/min/100ml (saline vehicle controls, Chapter 2, n = 9). The similar ~30% decrease in values observed here on the Bruker compared to the Agilent (and literature values) suggested potential issues with a) labelling efficiency and/or b) the repetition time (TR) parameter used during multi-T1 acquisition, both of which warranted investigation.

**Labelling Efficiency - Inversion Pulse Bandwidth and Shape**

Given the suspected sub-optimal labelling efficiency of the standard- and BCSFB-ASL methods on the Bruker 9.4T system, we aimed to optimise the inversion pulse bandwidth (BW) and shape. Utilising a higher inversion BW provides improved labelling due to decreased sensitivity to off-resonance effects that occur in the body of the mouse where labelling of blood takes place. However, as a trade-off, this higher BW can impact hardware radiofrequency transmit power limits, and SAR limits. On the other hand, lower BW values can result in an increased susceptibility to off-resonance effects leading to suboptimal labelling, despite placing a lower burden on hardware and fewer concerns regarding SAR limits. It has been shown that at high field strengths, a high BW is required to overcome these inhomogeneity effects, e.g. BW ≥ 15 kHz at 9.4T.

The default Perfusion_FAIR_EPI sequence utilises a 5 kHz inversion pulse BW, with a “calculated” shape (calc): an adiabatic full-passage (AFP) RF pulse shape with upward frequency sweep. ASL experiments on the Agilent 9.4T system utilised a hyperbolic secant...
(sech) shape inversion pulse with a 20 kHz BW. We investigated the effects of increasing the inversion pulse BW from 5 to 20 kHz, using both calc and sech shapes, in order to determine the optimal shape and BW combinations for the experiments (Figure 5). A cortical CBF baseline using 20 repetitions of standard-ASL was obtained in 3 mice, using the different BW-shape pairings (i.e. varying BW using a sech shape, and then varying BW with a calc shape, in the same subjects). The following MRI parameters were used for standard-ASL: TI = 2000 ms, TR = 5000 ms, TE = 20 ms.

Figure 5 highlights that at BW values of 5 and 10 kHz, we observe no significant differences in perfusion values obtained. However, at higher BW values required to overcome the field inhomogeneity effects, e.g. 15 and 20 kHz, cortical CBF values obtained with sech curves were distinctly lower than their calc counterparts. Paired 2-tailed t-tests revealed that the calculated shape pulses provided cortical CBF values which were in line with the previous measurements \(^1,^{122,138}\), as well as being significantly higher than those obtained using sech shapes, at 15kHz (p = 0.009), and 20 kHz (p = 0.029). The lower BF values obtained with a sech approach were also 2-3 times lower than CBF values described in the literature, as well as our own measurements obtained on the Agilent system with this exact BW-shape pairing (20 kHz, sech shape) \(^1,^{122,138}\).

To further investigate the underlying reasons for the sech pulses failing at higher bandwidths, we compared the inversion pulse amplitude (Figure 5b) and duration (Figure 5c), using the different bandwidth-shape combinations. At matching BW values, amplitude and duration values were identical (fixed) across the 4 subjects. We found that inversion pulse amplitude increased with BW. Amplitude was shown to increase linearly with bandwidth, and independent of pulse shape, i.e., sech and calc pulses gave identical amplitude values at each BW. Given that amplitude was not affected by pulse shape, we excluded amplitude differences as an underlying cause of the differences in inversion pulse efficiency.

Pulse duration decreased (non-linearly) with bandwidth for both sech and calc pulses. Importantly, calc pulses displayed consistently longer inversion pulse durations than sech curves, by a factor of 3.86. At higher BWs, sech pulses have a duration of 1.2 s (15 kHz) and 0.9 s (20 kHz) (Figure 5c).
Consider a rotating frame of reference for a spin system where there is a magnetization vector ($M$), aligned with the static magnetic field ($B_0$). When applying an oscillating magnetic field ($B_1$) to achieve magnetization inversion using an RF pulse, the frequency at which $B_1$ is applied is varied. For an adiabatic pulse, the frequency ($f$) begins far below the Larmor frequency ($f << \omega$), and then is swept through frequencies, eventually reaching omega ($f = \omega$) and then proceeding to values beyond omega ($f >> \omega$). This rotating frame is locked into sync with the $B_1$ field at the applied frequency ($f_1$). If $f_1$ is not equal to the initial frequency, $f_0$, at any point during the ramping of frequencies, then there will be a residual component of longitudinal magnetisation, $B_z'$. As a result of having a $B_1$ vector and $B_z'$ vector, there is a resultant $B_{\text{eff}}$.  

**Figure 5 - Inversion pulse bandwidth and shape (n = 3).** Varying inversion pulse bandwidth from 5-20 kHz, using different inversion pulse shapes: sech (blue) and Bruker’s calculated “calc” shape (red). Measuring cortical CBF (a), as well as post-hoc investigation into inversion pulse amplitude (b) and duration (c). Error bars: ± standard deviation (20 repetitions).
vector from their vectorial addition in the rotating frame. When \( B_1 \) is applied off-resonance, then \( M \) will precess around \( B_{\text{eff}} \).\(^{179}\)

Building from this, adiabatic inversion pulses operate under the adiabatic passage principle\(^{180,181}\): a magnetization vector initially parallel to \( B_{\text{eff}} \) follows the direction of \( B_{\text{eff}} \), provided that \( B_{\text{eff}} \) does not change its direction significantly during one rotational period of the magnetization about \( B_{\text{eff}} \), i.e. the rotation of \( B_{\text{eff}} \) should be much slower than the amplitude of \( B_1 \). Therefore, the ramping of \( B_1 \) frequencies should be conducted slowly enough, so that the magnetization precesses many times around \( B_{\text{eff}} \) during the process. Then \( M \) will follow \( B_{\text{eff}} \), precessing in a tight cone around it, and eventually result in complete 180° inversion in the z-axis\(^{179}\).

In order to satisfy the adiabatic condition, an adiabatic inversion pulse requires a relatively long pulse width for a slow, gradual rotation of \( B_{\text{eff}} \), and a high \( B_1 \) amplitude\(^{179–181}\). Given that at each BW, pulse amplitudes are identical for sech and \( \text{calc} \) inversion pulses (Figure 5b), it is likely that at higher BWs, sech inversion pulses fail due to having frequency sweeps that are too fast. These fast frequency sweeps may increase the rotational rate of \( B_{\text{eff}} \) so that the amplitude of \( B_1 \) is no longer large enough to facilitate adiabatic inversion, thereby violating the adiabatic condition and leading to an incomplete inversion\(^{179–181}\).

We sought to find a solution to the sub optimal labelling efficiency resulting from this adiabatic violation, without requiring pulse sequence programming. Software limitations prevent the experimenter from manually lengthening pulse durations at higher BWs, and as such, limits effective inversion using higher BW sech pulses which were only able to be applied with fixed, shorter pulse durations. On the other hand, the previously obtained water phantom dataset (Figure 2) highlighted that lower BWs yield non-zero \( \Delta M/M_0 \) values, thereby also limiting the use of lower BW pulses. The optimal choice moving forward was to utilise higher BW calc pulses. Unlike the optimisation of the inversion pulse BW, we see here that optimising and validating the ASL measurements on the Bruker system could not be achieved solely by reusing parameters which had previously worked well on the Agilent system, namely the inversion pulse shape and its set bandwidth.
**Single-TI BCSFB-ASL comparison between systems**

Thus far, several steps have been taken to optimise the BCSFB-ASL acquisition, namely the partial-FT parameter choice (“off”), as well as the labelling efficiency, i.e., choice of inversion pulse shape (“calculated”) and inversion pulse bandwidth (20 kHz). In order to determine the improvement in measurement precision (SNR) on the Bruker 9.4T system compared to the originally characterised method on the Agilent system, we compared baseline measurements of BCSFB-ASL obtained at a single-TI. The following parameters were used: TE = 220 ms, TI = 4000 ms, TR = 12000 ms, FOV = 20 mm × 20 mm, matrix = 32 × 32, slice thickness = 2.4 mm, n = 1, repetitions = 20.

Upon comparing single-TI baselines of relative BCSFB-mediated water delivery (Figure 6), we demonstrate a marked improvement in precision on the Bruker system. This can be observed from visual inspection of the two baselines and is further supported by a ~2-fold reduction in standard deviation of the measurement across the 20 repetitions in 2 biologically independent mice: [Bruker = 0.22 (n = 1), Agilent = 0.42 (n = 1)].

![Figure 6 - Optimised SNR with BCSBF-ASL](image)

**Figure 6 - Optimised SNR with BCSBF-ASL.** Comparison of a 20-minute-long, fixed single-TI, baseline BCSFB-ASL measurement acquired previously in the Agilent system (blue dashed line, n = 1 mouse), compared to the optimised methodology on the Bruker system (red line, n = 1 mouse). Parameters: TI = 4000 ms, TR = 12000 ms, TE = 220 ms, FOV = 20mm × 20mm, matrix = 32 × 32, slice thickness = 2.4 mm. Optimised/distinct parameters: inversion pulse shape (calc) and BW (20 kHz), partial-FT (“off”).
The improvement in BCSFB-ASL measurement precision observed thus far is beneficial for future pharmacological MRI (phMRI) measurements, similar to those conducted in Chapter 2. In previous work, *in-vivo* baseline measurements of BCSFB function, prior to administering a pharmacological/gas challenge, were found to have high levels of noise in some subjects. In turn, this impacted sensitivity to detecting any challenge-induced modulation. By improving the BCSBF-ASL single-TI imaging paradigm here for dynamic time course capture, future phMRI experiments may be able to more sensitively and precisely capture pharmacological modulation, and as such, may be able to be sensitive to changes induced by e.g. hypercapnia, where we observed high levels of noise in the BCSFB-ASL dataset.

**Multi-TI Buxton kinetic modelling – after optimising labelling efficiency**

Following the changes to the default Perfusion_FAIR_EPI sequence described thus far, we again utilised a multi-TI ASL approach with Buxton modelling to test the validity of the optimised standard- and BCSFB-ASL measurements.

From the multi-TI data obtained at TE = 20 ms (TR = 12000 ms), an absolute cortical CBF value of 240 ml/min/100g was extracted (n = 3, 20 repetitions at each TI per subject, Figure 7). This value is in line with the previous measurements on the Agilent 9.4T system and the literature\(^1,12,13,8\), which strongly indicates that the steps taken to optimise labelling efficiency were successful. Thus, this provides confidence in the optimised Perfusion_FAIR_EPI sequence to accurately measure cortical CBF, which can be extended to using a single-TI approach.

Multi-TI BCSFB-ASL measurements obtained at TE = 220 ms (TR = 12000 ms) provided a BCSFB-mediated water delivery rate of 2.7 ml/min/100ml (n = 3, 20 repetitions at each TI per subject, Figure 7). Although we were again able to observe a kinetic curve for BCSFB-ASL that was distinct to standard-ASL, several issues with this kinetic curve were observed. Firstly, from visual inspection, there was a poor fit to the Buxton 2-compartment model despite having a recognisable shape, which may stem from the scaling of \(\Delta M\) signal (negative \(\Delta M\) values). As a result, the extracted absolute BCSFB-mediated water delivery value was an order of magnitude lower than values previously obtained on the Agilent system, as well as values from the literature\(^1,21,29\). Despite optimising the labelling efficiency, which improved the standard-ASL measurements, issues persisted in the BCSFB-ASL measurement.
Multi-TI Buxton kinetic modelling – Investigating repetition time (TR) and parameter ordering

Having improved the labelling efficiency of the Perfusion_FAIR_EPI sequence through changing inversion pulse bandwidth and shape, we decided to investigate the repetition time (TR) parameter as the root cause of the poor BCSFB-ASL multi-TI Buxton modelling and low perfusion value obtained. A constant TR value e.g. 12000 ms is used when moving from one TI value in the array to the next, irrespective of the TI. Although this TR was appropriate for single-TI BCSFB-ASL measurements, as well as multi-TI standard-ASL measurements, we hypothesised that this TR value may not be long enough for BCSFB-ASL multi-TI experiments. More specifically, a 12000 ms TR may be insufficient in allowing for full recovery of longitudinal magnetization ($M_L$) following an adiabatic inversion pulse. To investigate the effects of TR on the multi-TI acquisition, and whether a longer TR was required, we obtained BCSFB-ASL data in at varying TR values: 8000, 12000, and 16000 ms (n = 1, 10 repetitions, single scan session).
When comparing the T1 obtained from the IR fitting (M_C as a function of TI), we obtained an average T1_{CSF} value of 3.81 ± 0.03 s from the three different TR values used (data not shown). Despite the highly reproducible T1 value obtained, Figure 8 reveals that altering the TR has a marked effect on the resultant ASL kinetic curves.

Using a much shorter TR of 8000 ms resulted in ΔM values which were negative across all TI values, and therefore could not be used to appropriately fit a Buxton model, i.e., no physiologically relevant water delivery value was extractable (Figure 8a), reinforced by a poor goodness of fit (GOF, R^2 = -0.94). The GOF R^2 coefficient is used in regression analysis to quantify the discrepancy between expected (modelled) values compared to the acquired data that the model is fit to. As such, R^2 determines the quality of the fitting. An R^2 of 1 indicates that the regression predictions perfectly fit the data. Values of R2 below 0 indicates a poor quality of fitting which results from improper modelling.

When using the previous TR of 12000 ms, we see that the Buxton 2-compartment model can be fit to the data, and we are able to extract a BCSFB-mediated water delivery value of 12.7 ml/min/100ml (Figure 8b), which is markedly lower than the previous measures. Although allowing for a better fitting than TR = 8000 ms, GOF was still low (R^2 = -0.16).

**Figure 8 - Multi-TI BCSFB-ASL—varying TR (n = 1).** Averaged BCSFB-ASL multi-TI data used to fit a 2-compartment model. a) TR = 8000 ms, b) TR = 12000 ms, c) TR = 16000 ms. Error bars: ±SEM (n = 3).
Lastly, extending the TR to 16000 ms resulted in a Buxton curve with the best fit of the three TR values used (Figure 8c). From this fitting, we obtained a BCSFB-mediated water delivery value of 24.7 ml/min/100ml, which is now in line with BCSFB-mediated water delivery values obtained previously on the Agilent system. This suggests that we can obtain BCSFB-ASL multi-TI kinetic data and curve which fits well ($R^2 = 0.83$) only when the repetition time is set to a value long enough to allow for appropriate magnetization recovery between image acquisition steps, i.e. 16000 ms. $\Delta M$ and $M_C$ Images acquired from this successful multi-TI dataset are displayed in Figure 9, below.
Figure 9 – BCSFB-ASL multi-TI images: control (MC) and subtracted (ΔM). Images are displayed from a representative subject (n = 1), with each image being an average of the data obtained from ten repetitions at each TI value (10 repetitions).
We predict that TR values which are insufficiently long (e.g. 8000 ms, 12000 ms) prevent sufficient recovery of \( M_z \) to \( M_0 \). Instead, \( M_z \) recovers only partially to a false maximum (\( M_{0_{\text{app}}} \)), which has a lower magnitude than \( M_0 \). Then, when the next inversion pulse is applied, \( M_z \) is not inverted from \( M_0 \), but \( M_{0_{\text{app}}} \) instead. This effect will compound as a new \( M_{0_{\text{app}}} \) is reached after each TI repetition. Figure 10 displays a simulation of this effect across the arrayed TI values, including both control and labelled acquisitions, as conducted during the multi-TI acquisition, when using a constant TR vs TRec. This will impact the acquisition of data between repetitions, but also within a repetition, e.g. control and labelled imaged at each TI, as well as the transition between different TI values. As a more extreme demonstration of this effect, the lack of full recovery is most evident at TR = 8000 ms, where we observe an entirely erroneous and unstable dataset, which cannot be used to fit a Buxton kinetic curve (Figure 8a above). We have not simulated the effect of a truncated repetition time on the ASL (\( \Delta M \)) signal, as this requires complex simulations to describe the contributions from circulation, as well as more global effects across the mouse. The simulation presented here addresses the issue of insufficient magnetisation recovery empirically by providing a demonstration of changes within our imaging slice.
Figure 10 - Simulation: magnetisation recovery during multi-TI BCSFB-ASL. Two repetitions of the BCSFB-ASL multi-TI acquisition are simulated, including both control and labelled acquisitions in sequence. This $M_z$ simulation describes a multi-Ti inversion recovery sequence: $180^\circ$ inversion pulse ($0 \leq t < T_I$), followed by a $90^\circ$ pulse ($t = T_I$).

a) Constant repetition with TR = 12000 ms. Red dashed line displays reductions in $M_0$ achieved by insufficiently long TR and partial recovery of $M_z$.

b) Constant recovery with TRec = 12000 ms.

Optimised BCSFB-ASL multi-TI acquisition using constant repetition time (TR)

After observing the effects of an increased TR of 16000 ms in a single subject when acquiring a multi-TI dataset, i.e. an improved fit of the model and more accurate measures of water delivery across the CP (see Figure 8c above), we decided to test this approach in a separate
cohort of mice (n = 3). We used an identical approach as above: multi-TI BCSFB-ASL, 10 repetitions at each TI, TR = 16000 ms. Figure 11 displays the averaged data used to fit the Buxton 2-compartment model, which also highlights the good fit of the model following these optimisation steps (R² = 0.95). Averaging the water delivery rates obtained from individual subject fittings provided a mean value of 19 ± 2.9 ml/min/100ml, which is consistent the with previous measurement previously acquired by our group on the Agilent system 1.

This data reiterates that TR value has been optimised and highlights that altering factors contributing to labelling efficiency was not enough to overcome the poor model fit and low flow values extracted from previous BCSFB-ASL multi-TI data within this chapter.

![Figure 11 - BCSFB-ASL at TR = 16s. Group-averaged multi-TI BCSFB-ASL data, used to fit to a 2-compartment Buxton kinetic model. Error bars: ± SEM (n = 3).](image)

**Rostral vs Caudal BCSFB-ASL signal**

Previous work has shown that the CP tissue is predominantly found in the caudal end of the lateral ventricles, relative to more rostral regions 106. Given the known position of the CP (from histological analysis), Evans et al. showed there was a markedly lower BCSFB-ASL ΔM signal
from the rostral end, and therefore lower rates of BCSFB-mediated water delivery, as hypothesised ¹.

Here, as a further validation step using a similar approach as Evans et al., we compared the BCSFB-ASL signal from the caudal aspect of the lateral ventricles to more rostral positions in three subjects (n = 3)¹. We positioned the imaging slice at three distances from the caudal end: 0 mm (caudal, consistent with the default approach to BCSFB-ASL), +1 mm, and +2 mm (furthest rostral). Single-TI BCSFB-ASL scans were run with the following parameters: TI = 4000 ms, TR = 12000 ms, TE = 220 ms. When quantifying BCSFB-mediated water delivery at each of these positions (20 repetitions at each position), the volume of the lateral ventricles within each of the different imaging volumes was used for normalisation.

Figure 12 displays the quantified BCSFB-mediated water delivery values at the three positions. Using Pearson’s correlation calculation, it was shown that there was a significant negative correlation between water delivery rates and distance from the caudal LV (correlation = -0.7749, p = 0.014). As hypothesised, the quantified BCSFB-mediated water delivery value decreases as you move rostrally from the caudal end of the lateral ventricles (default position). Here, a paired 2-tailed t-test revealed that when comparing 0 to 2 mm, there was a trend towards a decrease in water delivery (p = 0.07, which may be a type II error influenced by low sample size).
This data reveals that there is a higher BCSFB-ASL ΔM signal arising from LV regions containing more CP tissue, reiterating that the BCSFB-ASL signal is co-localised with the CP tissue found within the lateral ventricles, thus providing further confidence in the source of the BCSFB-ASL signal from the optimised sequence on the Bruker system, i.e., the measured signal arises from BCSFB-mediated labelled blood water delivery to ventricular CSF.

**Optimising temporal resolution—single- and multi-TI**

**Single-TI**

Improving the single-TI temporal resolution would enable an increased precision and confidence in the ability of the BCSFB-ASL measurement to accurately determine baseline values of water delivery, which could be acquired in a reduced scan time. Furthermore, this increased temporal resolution boosts the sensitivity to detect changes in the signal from pharmacological modulation (as with Chapter 2 - phMRI). In order to improve the temporal

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Figure 12 - Rostral vs Caudal LV: BCSFB-mediated water delivery. BCSFB-mediated water delivery at three positions from the caudal section (0 mm) of the lateral ventricle, moving further rostral (1 and 2 mm). Pearson’s correlation coefficient = -0.7749, p = 0.014, n = 3.
resolutions of the BCSFB-ASL single-TI (TI = 4000 ms) acquisition, we compared and quantified data collected at different repetition time (TR) settings, as shown in Figure 13 (n = 4).

We used two different timing settings: a constant repetition time of 12000 ms (i.e., the default parameter used by Evans et al. \(^1\) and in Chapter 2 for BCSFB-ASL single-TI measurements), and a constant recovery time (TRec) of 6000 ms, using a fixed TE of 220 ms for both.

![Figure 13 - Temporal resolution optimisation: BCSFB-mediated water delivery. Comparison absolute BCSFB-mediated water delivery from different timing settings for data acquisition: [TR = 12000 ms] i.e. default, vs. [TRec = 6000 ms] (n = 4, 20 repetitions).](image)

The comparison of data acquired at TRec = 6000 ms to the default TR = 12000 ms in the same mice, using paired 2-tailed t-tests, indicated that changing the TR parameter has not significantly altered the absolute BCSFB-mediated water delivery value obtained (Figure 13). These results suggest that switching to a TRec of 6000 ms would not impact the absolute blood flow value obtained in each subject and, as such, may be a viable choice to improve the temporal resolution, and reduce the overall scan time. Using a TRec value of 6000 ms gives a TR\(_{eff}\) of ~10220 ms (i.e. TRec + TI + TE), which would enable each image to be captured 15% faster.
Previous experiments conducted to investigate repetition time (TR) and parameter ordering for the multi-TI acquisition suggested that a TR of 10000 ms may be a point where the ASL signal nulls: TR = 8000 ms yielded an erroneous result with negative ΔM values, compared to TR = 12000 ms which provided ΔM values that are more in line with a typical kinetic curve (Figure 8). In the single-TI experiments described here, it is likely that using a TReff ~10000 ms was viable due to a difference in parameter ordering between the single-TI acquisition, compared to when the multiple TIs have been arrayed within the same scan. A multi-TI acquisition using a constant TR of 10000 ms is likely insufficient time for adequate recovery of Mz to return to M0 for early inflow times (e.g. values of TI ≤ 2750 ms), but is sufficient for a (fixed) TI value of 4000 ms. Thus, the use of TRec = 6000 ms in single-TI BCSFB-ASL experiments provides an effective means of data acquisition with improved temporal resolution and scan times.

Multi-TI

We also aimed to improve temporal resolution for the multi-TI approach on the Bruker system. Here, we compared the usage of two different, constant recovery time (TRec, as opposed to repetition time) options: TRec = 12000 ms, and 8000 ms. Multi-TI BCSFB-ASL data were collected in 4 mice (n = 4, 10 repetitions), with both TRec = 12000 ms and 8000 ms collected within the same scan session, per subject. We aimed to assess whether increasing the temporal resolution by decreasing TRec would produce multi-TI datasets which i) enable high quality fitting of the Buxton kinetic model (high GOF), and ii) enable the extraction of identical BCSFB-mediated water delivery rates.

Our data revealed that using a TRec of 12000 and 8000 ms produced two very similar kinetic curves, from visual inspection (Figure 14), both of which with a high GOF ([TRec 12000 ms: R² = 0.86] and [TRec 8000 ms: R² = 0.73]). However, at the lower TI values (e.g. 200 and 750 ms), using a TRec of 8000 ms produced ΔM/M0 values which had increased levels of noise and further deviation from the model relative to the acquisition with TRec = 12000 ms (Figure 14 a, b).

From the averaged data collected at TRec = 12000 ms (Figure 14a), a BCSFB-mediated water delivery rate of 22.5 ± 4.2 ml/min/100ml was extracted, compared to the value of 26.6 ± 3.9 ml/min/100ml from using a TRec of 8000 ms (Figure 14b). T1CSF extraction from these data
revealed similar values of $3.64 \pm 0.14$ s when $T_{Rec} = 12000$ ms was used, and $3.61 \pm 0.18$ s when $T_{Rec} = 8000$ ms was used. Paired, 2-tailed t-tests revealed that neither the water delivery values, nor the $T1_{CSF}$ values obtained were significantly different ([BCSFB-mediated water delivery: $p = 0.34$], [T1$_{CSF}$: $p = 0.50$]). This suggests that, using a lower $T_{Rec}$ value of 8000 ms provides similar (non-significantly different) outputs of water delivery and $T1_{CSF}$ values.

![Figure 14 - Multi-TI BCSFB-ASL– varying recovery time ($T_{Rec}$): a) $T_{Rec} = 12000$ ms, b) $T_{Rec} = 8000$ ms. Error bars: ±SEM (n = 4).](image)

For the collection of multi-TI data, using a $T_{Rec}$ setting adjusts $T_{Reff}$ in a manner which prevents shorter TIs from having a TR which is longer than required (i.e. improved temporal resolution), by providing lengthened $T_{Reff}$ values for longer TIs to ensure maximal $M_Z$ recovery. In terms of reducing scan time, a single repetition of the above dataset takes ~3.7 mins to acquire (7 TI values, control and labelled images) using a constant TR of 16000 ms. Using a constant $T_{Rec}$ of 12000 ms would reduce this to ~3.5 mins, with a marked improvement to ~2.6 mins with a $T_{Rec}$ of 8000 ms. Given that the $T_{Rec}$ options provide not only faster scan times, but also identical extracted parameters ($T1_{CSF}$ and water delivery values) as using a constant TR option, the $T_{Rec}$ options can be considered for future use.

**Improving SNR - TE = 180 ms**

A reduction in TE was piloted for the purposes of improving SNR in the single-TI measurements. This optimisation of TE would enable the detection of more signal overall,
whilst maximising signal contribution from labelled water crossing the BCSFB into ventricular CSF, whilst maintaining a negligible contribution from grey and white matter tissues (as is currently the case with 220 ms\(^1\)). Assuming the T2 values of grey matter blood (30 ms\(^{121-123}\)), tissue (38 ms\(^{121-123}\)), and ventricular CSF (300 ms\(^{124}\)), using TE = 180 ms will capture approximately 0.25%, 0.88%, and 55%, respectively, of the theoretical magnetization at TE = 0 ms. Hence, we hypothesise that the signal arising from the grey matter and blood will be nulled due to their approx. 10-fold shorter T2 values.

Single-TI (TI = 4000 ms) data were obtained in 4 subjects (20 repetitions each) using different acquisition schemes with varying TR/TE combinations: default [TR = 12000, TE = 220 ms], A [TR = 12000, TE = 180 ms], and, given the improvement in temporal resolution observed previously, B [TRec = 6000, TE = 180 ms]. From these data, \(\Delta M\) (the ASL signal) values were extracted from ROIs drawn on the i) lateral ventricles, ii) cortex, and iii) background. We first sought to determine whether a shorter TE would yield an increase in \(\Delta M\) (higher ASL signal). In addition, cortical \(\Delta M\) signals were compared to the background \(\Delta M\) (air), to investigate whether partial volume effects from grey and white matter were contributing to the signal.
Table 1 - Comparison of cortical and background ΔM (A.U.) signal, as well as the corresponding standard deviation (Stdev, A.U.), using three different acquisition schemes: default: [TR = 12000 ms, TE = 220 ms], A: [TR = 12000 ms, TE = 180 ms], and B: [TRec = 6000 ms, TE = 180 ms]. Paired, 2-tailed, t-tests were conducted to compare cortical and background delta ΔM (A.U.) signals. (n = 4, 20 repetitions each).

From this data, we observe an approx. 2-fold increase in the ΔM signal from the lateral ventricles when lowering TE to 180 ms. This increase in LV signal was significant for combination B where TRec = 6000 ms (p = 0.024), and bordered significance for A where TR = 12000 ms (p = 0.061). Furthermore, using the default parameters, cortical ΔM was shown to have a minimal, 7.8 % contribution relative to the LV ΔM. Cortical contributions were shown
to be similar for protocols using lower TE values: [A: 7.1 %] and [B: 8.1 %]. Importantly, we measure a marked increase in the cortical ΔM signal, relative to the background ΔM signal, when lowering the TE from 220 to 180 ms (Table 1), which was found to be significant under conditions A (p = 0.012) and B (p = 0.014). Although the cortical signal was higher than background when using the default TR/TE combination, this was found to be non-significant (p = 0.098).

Ultimately, when decreasing the echo time, the 2-fold increase in the ΔM signal in the lateral ventricles is beneficial in boosting SNR. However, given the statistically significant nature of the signal increase in the cortex relative to the background, this data suggests that lowering the echo time used in BCSFB-ASL introduces potential contributions from partial volume effects. A limitation of this work is that various echo time values in the range of 180 – 220 ms were not investigated. Thus, future work will aim to conduct further optimisation to lower the echo time incrementally to in a manner which identifies the TE value which preserves the increases in signal observed at the lateral ventricles, without the presence of confounding partial volume effects from surrounding brain tissue.

**Intraventricular injection pilot**

We conducted a pilot study to optimise intra-ventricular injections in a single mouse (n = 1). As a useful by-product of these injections, we were able to conduct experiments to further characterise the source of the ΔM signal on the optimised protocol. A well-recognised side effect ~24 hours after the intra-ventricular injection surgical procedure is the accumulation of fluid in the scalp directly above the brain (e.g., Figure 1b from Lodhia et al., 2006). This is the result of an innate inflammatory response from cutting through the scalp, introducing a needle into the brain and suturing. The resultant oedemic region may have a long-enough T2 to measure a control/labelled signal at an ultra-long TE of 220 ms, but importantly, this fluid will be void of labelled blood water delivery owing to the lack of associated vasculature. Therefore, this provided an ideal experimental condition to determine whether the direct effect of the labelling and control inversion pulses is identical on the imaging slice in an in-vivo setting. I hypothesised that there will be a negligible ΔM signal in the fluid-filled region, i.e., no detectable BCSFB-ASL signal.
Here, we collected data using BCSFB-ASL, with the imaging slice centred aligned to capture both the LV and the oedemic region, with the following parameters: TE = 220 ms, TR = 12000 ms, single-TI (4000 ms), 20 repetitions, FOV = 20 mm × 20 mm, matrix size = 128 × 128, slice thickness = 2.4 mm. Notably, a higher resolution in-plane readout was trialled for the BCSFB-ASL measurement, so that we could identify regions of ASL signals at the ultra-long TE with higher spatial precision.

Coronal anatomical images shows the appearance of the inflamed region under the scalp following surgery (Figure 15a,b). In the BCSFB-ASL averaged control images (Figure 15c), a signal can be observed from the fluid under the skin. However, as hypothesised, there is no signal present in the subtracted (ΔM) image (Figure 15d). ROI-based analysis of the oedemic region revealed an averaged, normalised ΔM signal (ΔM/Mc) value of 0.002, which was significantly lower than the lateral ventricular ΔM/Mc value of 0.041 (p = 2×10^{-6}).

This result highlights that despite seeing a prominent ultra-long TE signal in the control images from the fluid-filled, inflamed region, there is a negligible BCSFB-ASL ΔM signal. Therefore, this indicates that the slice selective and global/non-selective inversion pulses have an identical effect on the imaging slice, and as such, the ΔM signals observed from the lateral ventricles derive from labelled blood water. Furthermore, this data has successfully been acquired at a higher in-plane resolution compared to prior applications of BCSFB-ASL. As such, we are able to resolve and characterise ASL signals arising from water delivery at the lateral ventricles, and compare this to the signals obtained from distal fluid- or CSF-rich spaces, such as the oedemic region observed here.
Figure 15 - Intraventricular injection pilot for BCSFB-ASL signal source validation (n = 1).

a) Pre-surgery coronal anatomical reference image (example).

b) Post-surgery coronal anatomical reference image, with identical geometry to (a).

c) Post-surgery BCSFB-ASL averaged control image.

d) Post-surgery BCSFB-ASL averaged ΔM image.

e) Post-surgery BCSFB-ASL averaged ΔM/Mc signals from lateral ventricles (LV) and inflamed scalp (oedema). Error bars: ± SEM (i.e. stdev/(√20 repetitions)).
**d. Conclusion**

In this chapter, we successfully developed and characterised the BCSFB-ASL methodology on the Bruker 9.4T system, utilising the improved hardware and software for optimisation. We first optimised the labelling parameters, starting with a water phantom and moving onto *in-vivo* mouse brain imaging. Subsequently, we improved the temporal resolution of the BCSFB-ASL methodology and the SNR. Optimisation of SNR by lowering the TE can be a focus of future studies, with a greater emphasis on maximising LV signal whilst minimising PVE contributions. Furthermore, the validation reinforced that BCSFB-ASL on the Bruker system was still detecting BCSFB-mediated water delivery, as evidenced by comparing the LV signal to CP-deficient regions with a long T2 (CSF, oedema). Importantly, the optimisation of single- and multi-TI approaches ensure that future experimentation will have increased precision, whilst minimising scan times. Lastly, data acquired at a higher in-plane resolution in an IVH model pilot suggests the possibility of investigating BCSFB-ASL signals in other brain regions with greater spatial precision than the previously used lower resolution readouts.
4. Chapter 4 – Brain-wide CSF spaces

a. Introduction

CP tissue in the mouse brain is found only within the cerebral ventricles, with a predominant distribution within the caudal lateral ventricles and upper 3rd ventricle\textsuperscript{106,183}. Given the location of the CP tissue, the application of the BCSFB-ASL method has thus far has been centred on assessing labelled arterial blood water delivery from blood to CSF at the lateral ventricles, as a surrogate measure of BCSFB function in the lateral ventricles. Recently, Petitclerc \textit{et al.} employed a multi-PLD, multi-echo ASL protocol to characterise the locations and dynamics of blood-CSF water exchange in the human brain\textsuperscript{132}. This work indicated that, in the human brain, the long-TE ASL signal is distributed in other brain regions beyond that of the lateral ventricles. The long TE ASL signal can be observed in the subarachnoid space (SAS) around the cortical surfaces of the brain (Figure 1), reflective of potential blood-CSF water exchange sites other than the BCSFB. This unexpected observation suggests that there may be alternative routes by which labelled blood water can be delivered to CSF that do not involve the CP (for example transfer across the arterial pial vessels in the subarachnoid space). This observation then presents two key questions: i) can we reproduce this finding and capture the transfer of labelled blood water into subarachnoid CSF in the anaesthetised rodent brain; ii) might there be contributions to the putative BCSFB-ASL signal in the lateral ventricles from labelled blood water that has not been delivered via the BCSFB (which would reduce the specificity of the measurement as a non-invasive surrogate of BCSFB function).
Figure 1 - Ultra-long TE ASL signal in human brain – example dataset. The signal originating from each of the three compartments is shown separately (Blood, Gray Matter, CSF). Maps are given for three slices, one intersecting the circle of Willis (top), one intersecting the choroid plexus (middle) and one higher in the brain (bottom). Different time points are shown with increasing pCASL labelling duration (LD)/post-labelling delay (PLD) combinations from left to right. Ultra-long TE ASL signal is present in choroid plexus within the lateral
ventricles, and the subarachnoid space around the cortex (bottom panel). Taken from Petitclerc et al. [Figure 6132].

Following the observation that the ultra-long TE ASL signal was distributed in subarachnoid regions in the human brain, here we sought to further characterise the contributing sources and kinetics of the BCSFB-ASL signals in the mouse brain. Multi-TI BCSFB-ASL data was obtained in the SAS of the rat brain, and in CP tissue-deficient ventricular regions in the mouse brain, namely the rostral LV and bottom 3rd ventricle (Figure 2, Figure 3 and Figure 4). This investigation seeks to determine whether reliable water delivery values in these CP-deficient regions can be obtained, in a similar fashion to the caudal LV and top 3rd ventricle which contain CP tissue (Figure 2). Furthermore, these data will inform on other possible factors contributing to the appearance of long TE ASL signals in regions beyond that of the caudal lateral ventricles for example: routes of non-BCSFB mediated labelled water entry, possibilities of “direct vessel exchange” (transfer across proximal pial vessels) or “vessel contamination” (intravascular signal contributions), as well as the involvement of rapid CSF mixing.
Figure 2 - Allen mouse brain atlas - choroid plexus tissue (green) location overlaid with:

a) Lateral ventricles (grey).
b) 3rd ventricle (grey).

**b. Methods**

**Data collection**

All the data collected towards the aims in this chapter were collected by me.

**Animal preparation and anaesthetic induction/maintenance**

All animal procedures were performed under the UK Home Office Act (Scientific Procedures, 1986). C57/BL6 WT mice (provided by Charles River Laboratories) were used for the purposes of angiography, and comparison of CP-rich and CP-deficient CSF regions with the multi-T1 BCSFB ASL approach. Anaesthesia was induced using 2% isoflurane in 0.8L/min medical air and 0.2 L/min O₂. Following induction and weighing, the mice were placed into the MRI cradle with bite bar, nose cone and ear bars to ensure a well secured position of the mouse head to minimise motion during the data acquisition. Eye ointment was also applied. During the acquisition, anaesthesia was maintained by reducing isoflurane concentration to 1.5% in 0.4 L/min medical air and 0.1 L/min O₂. Temperature and breathing rate were monitored throughout all the experiments using a rectal probe and a respiration pad (SA Instruments). Mouse temperature was maintained at 37 ± 0.5°C using a combination of heated water tubing and warm air flow during both the induction and data acquisition stages.

In addition, Wistar Kyoto rats (WKY, provided by Envigo) were used for the study of the BCSFB-ASL signal in the subarachnoid space. WKY rats underwent an equivalent set-up to mice, with anaesthetic induction using 4% isoflurane in 0.8 L/min medical air and 0.2 L/min O₂, and anaesthetic maintenance at 2% isoflurane in 0.4 L/min room air and 0.1 L/min O₂.

**Magnetic resonance imaging (MRI) protocols**

Images were acquired on a 9.4 T Bruker imaging system (BioSpec 94/20 USR) with a horizontal bore and 440 mT/m gradient set with an outer/inner diameter of 205 mm /116 mm respectively (BioSpec B-GA 12S2), 86 mm volume coil and a four-channel array rat or mouse brain surface coil (RAPID Biomedical GmbH) for the transmission and the reception of the RF signal, respectively. For imaging of rats, the centre of the volume coil was positioned caudally 3 cm from the iso-centre of the magnet to ensure a good labelling efficiency of the global inversion pulse during the flow-alternating inversion recovery (FAIR)-ASL acquisition.
Anatomical reference scans

A T2-TurboRARE sequence (fast-spin echo, Paravision v6.0.1) was used to collect sagittal and coronal anatomical reference images to clearly visualise the location of the CSF compartments in the mouse brain, such as the lateral ventricles (caudal and rostral regions), 3rd ventricle (top and bottom regions), and the subarachnoid space (above the rostral cortex). Sequence parameters were: (field of view (FOV) = 20 mm × 20 mm; matrix size = 256 × 256; RARE factor = 8; effective echo time (TE) = 33 ms; repetition time (TR) = 2500 ms. This sequence was also used for the rat brain, with an alteration in the FOV to 30mm × 30mm.

Sagittal anatomical reference images were used to position the axial anatomical reference imaging slice and the ASL imaging slices in mice (9 × 1 mm) and rats (12 × 1 mm). In previous chapters, coronal anatomical reference images were manually positioned to enable the segmentation of the LV and a volume calculation. In this chapter, coronal imaging slices were positioned to enable the visualisation of the selected aforementioned CSF spaces in mice (1 × 0.4 mm), and the rat SAS (1 × 0.4 mm)

Angiography

Aim: To investigate whether large arteries are directly proximal to the caudal aspect of the lateral ventricles in the mouse brain (where BCSFB measurements have so far been concentrated owing to the relatively high concentration of CP tissue in this region).

In the mouse brain (C57/BL6, female, 6 months old, n = 1), angiography was conducted to qualitatively assess large vessel colocalization with the lateral ventricles. A TOF 3D FLASH sequence was used, with the following parameters: averages = 2, slice thickness = 10 mm, matrix size = 256 × 256 × 96, and FOV = 20 mm × 20 mm × 10 mm, TE = 3.15 ms, TR = 18 ms. From individual slices in the 3D dataset, images corresponding to equivalent slices in the coronal T2-weighted anatomical reference images were selected for comparison.
Multi-TI BCSFB-ASL

As described in Chapter 3, the optimised multi-TI BCSFB-ASL approach was used for the measurement of BCSFB-mediated water delivery. The BCSFB-ASL imaging protocol was based on the optimised perfusion sequence offered by Paravision v6.0.1 (Bruker software) using a flow-alternating inversion recovery (FAIR) acquisition with a single slice, single shot SE-EPI readout, 20 mm slice-selective width, 20kHz “calculated” inversion pulse, and a global labelling pulse, across all the experiments. Field inhomogeneities were improved through pre-scan automatic shim adjustments. 6 dummy scans were included prior to acquisition, along with having partial-FT off (1.0, 1.0).

In order to characterise the BCSFB-ASL signal in CSF spaces within the brain with a higher degree of spatial precision, we use a higher in-plane resolution and narrower imaging slices for the BCSFB-ASL readout (Figure 3 and Figure 4 below showing high resolution ASL images), e.g. [matrix = 128 × 128, FOV = 20 mm × 20 mm], compared to previous applications in the mouse brain which looked at BCSFB function at CP tissue distributed at the caudal end of the lateral ventricles [matrix = 32 × 32, FOV = 20 mm × 20 mm]. To avoid ghosting artefacts for higher resolution BCSFB-ASL experiments, we use Paravision v6.0.1’s built-in receiver gain (RG) and reference power (RP) calculation at a lower echo time (TE = 53 ms) prior to running the EPI sequence at the ultra-long TE (TE = 220 ms). Without this correction, the EPI, and RG and RP calculations, would be conducted at TE = 220 ms.

Rat brain multi-TI – subarachnoid space

Aim: To investigate the presence of ultra-long TE ASL signals in the subarachnoid space (SAS), which were previously observed in the human brain, we characterise the kinetics of the SAS-localised signal observed in the rat brain.

Here, we used a Wistar Kyoto rat model (WKY, provided by Envigo), which has a more prominent SAS volume than the mouse brain. T2-weighted anatomical images guided the identification of SAS above the rostral cortex (Figure 3 a,b). A multi-TI BCSBF-ASL approach (as described in Chapter 1 - Section I) was applied to characterise the kinetics of the BCSFB-ASL signal at the SAS (Figure 3c, d). Applying the 2-compartment Buxton kinetic model to the
SAS region now aims to characterise the kinetics of the delivery of labelled arterial water from the vascular compartment into the CSF within the SAS, through mechanisms which are yet to be elucidated within the literature (see Chapter 1 – Section e). An initial pilot study was conducted using a WKY rat (male, 10 weeks old, n = 1). Subsequently, multi-TI experiments were conducted in a larger WKY cohort (male, 10 weeks old, n = 3). In the n = 3 dataset, for the purposes of exploratory analyses, τ and Δt were obtained by first averaging the ΔM/M0 values across the cohort, and then extracting a single Δt and τ value from the average kinetic curve.

BCSFB-ASL parameters: TE = 220 ms, repetitions = 10, slice thickness = 1 mm, matrix size = 128 × 128, FOV = 32 mm × 32 mm, TRec = 12000 ms, TI = [200, 750, 1500, 2750, 4000, 5000, 6000].

![Figure 3 - Pilot investigation into rat brain SAS-based BCSFB-ASL signal (n = 1). This figure displays the region across which the BCSFB-ASL acquisition is conducted, and the resultant ASL control and subtracted images.](image)

- **a)** Sagittal anatomical reference image (T2-weighted), depicting position of BCSFB-ASL imaging slice (green) to capture SAS region.
b) Coronal anatomical reference image (T2-weighted) showing SAS region.

c) SAS: BCSFB-ASL multi-TI control image, average at TI = 4 s (10 repetitions).

d) SAS: BCSFB-ASL multi-TI subtracted (ΔM) image, average at TI = 4 s (10 repetitions).

Mouse brain multi-TI - CP-rich vs deficient regions

Aim: to determine whether reliable BCSFB-mediated water delivery values could be obtained from CP-deficient CSF regions by characterising the kinetics of the long TE ASL signal within these regions. We also sought to explore the contributions of rapid CSF mixing, or other routes of labelled water delivery to CP-deficient regions, compared to their CP-rich counterparts.

We collected multi-TI BCSFB-ASL data in the mouse (C57/BL6, female, 6 months old, n = 6). Using T2-weighted anatomical images (Figure 4a,b), ASL imaging slices were positioned to include CP-rich regions (caudal lateral ventricles, top 3rd ventricle) and CP-deficient regions (rostral lateral ventricles, bottom 3rd ventricle), owing to the known distributions of the CP tissue

Multi-TI data obtained using a caudally positioned slice was used for the investigation of three regions: caudal LV (CP-rich), top 3rd ventricle, and bottom 3rd ventricle. A rostrally positioned imaging slice provided data used to assess the rostral LV (Figure 4). Rostral and caudal slices were obtained within the same scan session, and allowed paired comparisons (2-tailed, paired t-tests) to be made between i) rostral and caudal regions, and ii) top vs bottom 3rd ventricle regions.

Multi-TI BCSFB-ASL parameters were: TE = 220 ms, repetitions = 10, slice thickness = 0.8 mm, matrix size = 128 × 128, FOV = 20 mm × 20 mm, TRec = 12000 ms, TI = [200, 750, 1500, 2750, 4000, 5000, 6000 ms].
Figure 4 - Characterising BCSFB-ASL signal in CSF spaces. This figure displays the region across which the BCSFB-ASL acquisition is conducted, and the resultant ASL control and subtracted images. Images shown from representative subject (n = 1):
a) Sagittal anatomical reference image (T2-weighted), depicting position of BCSFB-ASL imaging slices to capture rostral (green) and caudal (red) regions of the ventricles.

b) Coronal anatomical reference images (T2-weighted) showing caudal and rostral slices, with overlaid CSF regions for characterisation: caudal lateral ventricles (red), top 3rd ventricle (blue), bottom 3rd ventricle (yellow), rostral lateral ventricles (green).

c) Caudal slice: BCSFB-ASL multi-TI control image, average at TI = 4 s (10 repetitions).

d) Caudal slice: BCSFB-ASL multi-TI subtracted (ΔM) image, average at TI = 4 s (10 repetitions).

e) Rostral slice: BCSFB-ASL multi-TI control image, average at TI = 4 s (10 repetitions).

f) Rostral slice: BCSFB-ASL multi-TI subtracted (ΔM) image, average at TI = 4 s (10 repetitions).

Image processing and analysis for ASL quantification

When analysing BCSFB-ASL images obtained with TE = 220 ms, a single region of interest (ROI) was drawn for each subject around the CSF-space we wished to investigate, e.g. SAS, top/bottom 3rd ventricle, rostral/caudal lateral ventricle. This ROI was drawn on the (control) FAIR image, and the mean voxel signal was calculated across the ROI. This was feasible given the higher resolution of the BCSFB-ASL images in this study compared to the work described in previous chapters. For each ASL image pair, the non-selective mean ROI value (M₀) was subtracted from the slice-selective (labelled) mean ROI value to provide the perfusion-weighted signal ΔM. In previous chapters, ΔM/M₀ underwent a correction to account for the ROI volume (12 voxels) and ventricular volume. However, this was not done within this chapter due to the high resolution BCSFB-ASL imaging which enabled a clearer identification of the selected CSF-spaces within the slice (e.g. Figure 3c,d and Figure 4c-f).

Repeated measures of ΔM and M₀ values were averaged for each TI providing [TI, ΔM] and [TI, M₀] datasets. A simple inversion recovery curve was fit to the [TI, M₀] data, permitting the extraction of T1 and M0 for each subject. The T1 of the CSF (T1_{CSF}) space was calculated from the control images acquired at TE = 220 ms. The [TI, ΔM] values were then used to fit the relevant Buxton model utilising the calculated T1 and M0 values. A 2-compartment adaptation of the Buxton Kinetic model was fit to multi-TI ΔM/M₀ data. The adaptation aims to account for transit effects and intraluminal spins more accurately,
ultimately allowing for the model to be utilised to describe the delivery of labelled blood water into the CSF (compartment), as opposed to extra-vascular brain tissue in standard-ASL.

The 2-compartment Buxton Kinetic model permits the extraction of 3 variables: the rate of labelled water delivery, as well as arrival time ($\Delta t$) and temporal length ($\tau$) of the tagged bolus of arterial blood water. In this chapter, multi-TI datasets were used to fit the model to extract all 3 parameters, thereby characterising the kinetic differences in the BCSBF-ASL signal between the selected CSF-spaces. In instances in which we aim to extract only the rate of BCSFB-mediated water delivery with a high degree of precision, a single parameter 2-compartment fit was used, i.e., fixing $\Delta t$ and $\tau$.

For the purpose of visual comparison of the averaged curves which describe the BCSFB-ASL kinetics in the various CSF spaces studied (SAS, top/bottom 3rd ventricle, rostral/caudal lateral ventricles) the values of $\Delta M/M_0$ values were averaged (e.g. Figure 8a,b and Figure 9a,d).

**CSF motion and rapid mixing**

**Aim: To determine potential contributions of CSF motion and rapid mixing to the BCSFB-ASL signal in CSF regions deficient in CP tissue.**

Multi-TI BCSFB-ASL data was obtained in the mouse brain ($n = 1$, 5 repetitions each) at high resolution (matrix $= 128 \times 128$, FOV $= 20 \text{ mm} \times 20 \text{ mm}$). These multi-TI scans had a fixed imaging slice thickness (1.00 mm) positioned at the rostral end of the lateral ventricles, with varying FAIR-ASL slice-selective inversion slab thicknesses. We first conducted the multi-TI scan with a low inversion slab thickness, i.e. 1.05 mm (minimum achievable), positioned at the rostral end of the lateral ventricles. Then, two more multi-TI scans were conducted with same rostral position of 1.00 mm imaging slice, but with incrementally increased inversion slab thicknesses: 1.50 mm and 2.00 mm (Figure 5).

The $\Delta M/M_0$ data obtained at each inversion slab thickness was used to fit a 2-compartment Buxton kinetic model. This multi-TI imaging protocol was not designed to quantify perfusion, but rather would provide information on back-and-forth CSF motion from the inversion slab to the imaging slice similar to that of the TimeSLIP approach $^{184}$. As such, the kinetic curve fittings were used to qualitatively compare extents of relative CSF mixing. Furthermore, by
using a 3-parameter fitting (flow, Δt, and τ), we were able to obtain values of arrival time of the labelled blood water into our imaging slice (Δt).

**Figure 5 - CSF motion and rapid mixing method using multi-TI BCSFB-ASL.** Fixed FAIR-ASL imaging slice thickness (1.00 mm) at the rostral end of the lateral ventricles. Increasing inversion slab thickness in separate multi-TI scans (1.05, 1.50, 2.00 mm).

**Statistical methods**

Multi-TI BCSFB-ASL datasets acquired to characterise differences in the kinetics of water delivery at CP-rich vs CP-deficient regions in the mouse brain were compared using paired, 2-tailed, Student’s t-tests (e.g. when comparing caudal LV vs rostral LV, and comparing top 3V vs bottom 3V (Figure 9)).
c. Results

Angiography

Recent work has presented evidence for rapid transfer of labelled blood water into subarachnoid CSF in the human brain using long TE ASL measurements\textsuperscript{132}. A possible explanation for this is the transfer of labelled blood water across the wall of the arterial pial vessels which are in direct contact with subarachnoid CSF.

Here, an angiography dataset (TOF 3D FLASH, Figure 6) was obtained in the mouse brain to qualitatively identify large vessels which may be colocalised with the lateral ventricles, and therefore potentially contributing to the BCSFB-ASL signal (via the delivery of labelled water to ventricular CSF across the wall of the vessel). We identified slices from the TOF 3D FLASH data which corresponds to slices in the T2-weighted anatomical images containing the lateral ventricle structures. Visual inspection revealed that arterial vessels are clearly depicted in the brain (TOF 3D FLASH bright signal), as expected (Figure 6). Importantly, despite one of these vessels (azygous cerebral vein) being close to the lateral ventricles, there were no obvious vessels found to be in direct contact with the lateral ventricles in the imaging region (Figure 6).

Figure 6 - Angiography for vessel identification. Displaying selected 3D TOF FLASH images corresponding to T2-weighted anatomical reference images in the caudal lateral ventricles.
Rat brain multi-TI – subarachnoid space

In the human brain, ultra-long TE ASL signals were found to be distributed in subarachnoid regions. To investigate the SAS-localised long TE ASL signals, we acquired long-TE ASL measurements in the SAS of a Wistar Kyoto (WKY) rat. We conducted a pilot study in a WKY subject (n = 1), within the ASL imaging slice positioned at the rostral cortex of the brain, where we identified a SAS region from sagittal and coronal images (Figure 3a, b). 2-compartment Buxton kinetic models were fit to multi-TI BCSFB-ASL data (see Chapter 1 – Section l) at high resolution (Figure 3c,d) (Figure 7a), which here, describes the delivery of labelled arterial water from the vasculature, into the CSF within the SAS. At this time, in-depth mechanisms and transporters responsible for potential water delivery are not known, but may be attributed to AQP-1-mediated transfer at the pial vessels \(^{185}\). On the other hand, potential delivery of labelled arterial water may occur as a result of initial BCSFB-mediated delivery of labelled water within ventricular choroid plexus tissue, which subsequently enters the brain-wide CSF pool and arrives at the SAS. And therefore, in the context of the kinetic model, the extracted arrival time (Δt) and the rate of water delivery will be reflective of these processes (which at this moment of time, have yet to be elucidated).

Buxton kinetic modelling of BCSBF-ASL data at the SAS permitted the extraction of the arrival time (Δt) for the labelled bolus of water into the SAS, which was found to be 2.31 s. BCSFB-ASL multi-TI data obtained from the caudal LV region (Figure 7b) from a representative WKY subject (n = 1, 10 repetitions) revealed distinct kinetics from visual inspection of the kinetic curve, as well as displaying lower Δt value of 1.12 s. The reasonable fit of the model to the SAS data provides evidence that we have captured the delivery of labelled water to the SAS with
a delayed arrival time, relative to kinetics at the caudal LV.

**Figure 7 - Pilot investigation into rat brain SAS-based BCSFB-ASL signal.**

a) **SAS**: averaged BCSFB-ASL multi-TI data alongside Buxton kinetic curve fit. Error bars: ± stdev of 10 repetitions.

b) **Caudal LV**: averaged BCSFB-ASL multi-TI dataset example, alongside Buxton kinetic curve fit. Imaging slice positioned at the caudal end of the lateral ventricles, obtained from another WKY rat (n = 1, 10 repetitions). Error bars: ± stdev of 10 repetitions.

Following the pilot in a single WKY subject, further investigation was conducted in 3 subjects (Figure 8). $M_c$ data from the SAS were used to fit an inversion recovery curve to extract average $T1_{CSF}$ (3.83 ± 0.21 s) and inversion efficiency (95% ± 1.6%), both of which are in line with values obtained from ASL analyses of CSF spaces throughout this work. However, BCSFB-ASL data from the SAS of these subjects displayed poor quality fits of the kinetic model and yielded highly variable $\Delta M/M0$ values (Figure 8a,c).

For the purposes of post-hoc, exploratory analyses, early TI values (<1.50 s, Figure 8b) with higher variability were excluded for the fitting. $\Delta t$ was extracted from the averaged multi-TI $\Delta M/M0$ data in Figure 8b, which revealed a $\Delta t$ value of 2.5 s, which was similar to that of the pilot study ($\Delta t = 2.31$ s), and markedly longer than arrival time for the caudal LV ($\Delta t = 1.12$ s).
Figure 8 - n = 3 investigation into rat brain SAS-based BCSFB-ASL signal (note different ΔM/M0 axes scales).

a) SAS: all multi-TI data, averaged ultra-long TE ASL multi-TI data alongside Buxton kinetic curve fit. Error bars: ± SEM (n = 3).

b) SAS: excluding early TI values, averaged ultra-long TE ASL multi-TI data from TI values < 1.50 s alongside Buxton kinetic curve fit. Error bars: ± SEM (n = 3).

c) SAS: example BCSFB-ASL multi-TI dataset (n = 1). Error bars: ± stdev of 10 repetitions.
Mouse brain multi-TI - CP-rich vs deficient regions

BCSFB functional signals were compared from regions rich and deficient in choroid plexus tissue within the mouse brain ventricles based on the Allen mouse brain atlas\(^{183}\) (Figure 2, Figure 4, and Figure 9). By fitting a 3-compartment Buxton kinetic model to multi-TI \(\Delta M/M_0\) values, these experiments aimed to: i) determine whether reliable BCSFB-mediated water delivery values can be obtained from CP-deficient regions, and ii) further characterise the kinetics of the BCSBF-ASL signal (ie. \(\Delta t, \tau\)) in these regions. Furthermore, we also attempt to explore the contribution of rapid CSF mixing, or other means of labelled water delivery to CP-deficient regions, compared to their CP-rich counterparts, i.e. rostral vs caudal lateral ventricles, top vs bottom 3\(^{rd}\) ventricle (Figure 9).

We observe a higher rate of labelled water delivery in regions more densely populated with CP tissue than CP-deficient regions, as evidenced by the extracted, mean rate of water delivery across the subjects ([caudal 56 ± 15, rostral 26 ± 13 ml/min/100ml, \(p = 0.01\)] and [top 3V 73 ± 12, bottom 3V 11 ± 6.3 ml/min/100ml, \(p = 0.0013\)]). This can also be observed through visual differences in the kinetic curves (Figure 9a (caudal LV vs rostral LV), Figure 9d for (top 3V vs bottom 3V), where CP-rich regions provide markedly elevated \(\Delta M/M_0\) values across the Tis.

However, the 3-parameter fit revealed no definitive changes in \(\Delta t\) for caudal vs rostral LV, or top vs bottom 3V ([caudal 0.24 ± 0.15 s, rostral 1.25 ± 0.63 s, \(p = 0.056, n = 6\)] and [top 3V 0.22 ± 0.50, bottom 3V -0.51 ± 2.53 s, \(p = 0.69, n = 6\)]) (Figure 9b, e). Following on from this initial analysis, we excluded subjects which had \(\Delta t\) values below 0 (i.e. not physiologically meaningful). Subsequent paired t-testing revealed that despite a trend towards lengthening of the \(\Delta t\) in CP-deficient regions, there were no statistically significant differences observed ([caudal 0.31 ± 0.12 s, rostral 1.45 ± 0.63 s, \(p = 0.074, n = 5\)] and [top 3V 0.66 ± 0.08, bottom 3V 1.60 ± 0.33 s, \(p = 0.073, n = 4\)]) (Figure 9c,f). Importantly, fitting quality for CP-deficient regions was noticeably lower, from visual observation, due to the lower overall signal (Figure 9a,d).
Figure 9 – Characterisation of BCSFB-ASL signal kinetics in CSF spaces:

a) Caudal vs rostral LV: averaged BCSFB-ASL multi-TI data alongside 3-parameter Buxton kinetic curve fit. Error bars: ± SEM (n = 6). Red: caudal data (marker) and corresponding fit (line). Green: rostral data (marker) and corresponding fit (line).

b) Caudal vs rostral LV: paired comparisons of Δt per subject (n = 6).

c) Caudal vs rostral LV: paired comparisons of Δt per subject after removing values of Δt < 0 (n = 4).

d) Top vs bottom 3V: averaged BCSFB-ASL multi-TI data alongside 3-parameter Buxton kinetic curve fit. Error bars: ± SEM (n = 6). Blue: top 3V data (marker) and corresponding fit (line). Yellow: bottom 3V data (marker) and corresponding fit (line).
e) Top vs bottom 3V: paired comparisons of Δt per subject (n = 6).

f) Top vs bottom 3V: paired comparisons of Δt per subject after removing values of Δt < 0 (n = 4).

g) Caudal vs rostral LV: paired comparisons of BCSFB-mediated water delivery per subject.

h) Top vs bottom 3V: paired comparisons of BCSFB-mediated water delivery per subject.

CSF motion and rapid mixing

Further investigation was conducted into the contribution of CSF motion and rapid mixing to the presence of the BCSFB-ASL signal in CP-deficient regions of CSF, such as the bottom 3rd or rostral ventricles. Specifically, whether CSF motion/rapid mixing may contribute to the appearance of the long TE ASL signal in these aforementioned regions (e.g. rostral LV, bottom 3rd etc.). We applied a high resolution, multi-TI BCSFB-ASL readout, with a fixed imaging slice thickness at the caudal end of the LV (0.8 mm), and increasing inversion slab thicknesses (1.05, 1.50, 2.00 mm).

Qualitative assessment of this data from visual observation highlights that we are able to observe the appearance of the ASL signal in a similar timescale to CP-rich regions (caudal LV, top 3V), i.e., an initial arrival time, rise and maximum at 3-4 s. As expected, as the imaging slice thickness was increased, ΔM/M0 values across the range of TIs decrease markedly, i.e., a reduced maximum ΔM/M0 as well as flatter Buxton kinetic curves overall. This suggests that CSF mixing contributions will likely impact our measurement of the BCSFB-ASL signal in CP-deficient region, as there is a marked contribution of inflowing spins from water molecules which arrive from outside the imaging slices in the BCSFB-ASL data.

Fitting the 3-parameter Buxton kinetic curves to the data to obtain arrival time (Δt) values revealed that arrival time increased with inversion slab thicknesses ([1.05 mm, Δt = 0.50 s], [1.50 mm, Δt = 1.25 s], [2.00 mm, Δt = 1.40 s]).
Figure 10 - CSF motion and rapid mixing. BCSFB-ASL multi-TI data obtained using a fixed imaging slice thickness (1.00 mm), with varying inversion slab thickness (1.05, 1.50, 2.00 mm). ΔM/M₀ data were used to fit the 2-compartment Buxton kinetic model to qualitatively assess CSF mixing contributions.

**d. Discussion**

The application of BCSFB-ASL thus far has been in the mouse brain to non-invasively study the function of the choroid plexus tissue primarily in the caudal lateral ventricles. Recent brain-wide application of a similar, ultra-long TE approach in humans has revealed the appearance of ASL signals in the subarachnoid space (SAS). Petitclerc et al. propose several reasons for discrepancy in signal co-localisation from the work conducted in the mouse brain. Firstly, they suggest that this signal may reflect genuine extra-ventricular blood-CSF water exchange related to the presence of aquaporin-1 (Aqp-1) water channels. Aqp-1 is present in high concentrations at the CP and plays a key role in CSF secretion. Recent work has identified Aqp-1 in the pial vasculature of rodents, i.e. at the SAS. However, in the mouse brain, Evans et al. did not observe the presence of signal beyond that of the ventricles. This
discrepancy may be influenced by biological factors, such as smaller SAS volumes or variable Aqp polarisation in mice. MR-imaging based factors, such as having a low imaging resolution in BCSFB-ASL, may also play a role.

To characterise the appearance of this signal in regions beyond that of the caudal lateral ventricles, we conducted several experiments to determine whether reliable water delivery rates values could be obtained, as well as further investigating the sources of the BCSFB-ASL signal, such as the involvement of non-CP mediated routes of labelled water delivery, as well as rapid CSF motion.

**Angiography**

Petitclerc et al. propose the possibility of a proximal pial vessel which contributes to the appearance of long TE ASL signals in these extra-ventricular regions via AQP-mediated transfer of labelled blood, which we term “direct vessel exchange” here, rather than by BCSFB-mediated transfer. As a preliminary investigation into whether vessel presence was ‘contaminating’ the BCSFB-ASL signal obtained from the caudal LV of the mouse brain, we obtained TOF 3D FLASH angiography data to locate large arterial vessels colocalised with the lateral ventricles. The data revealed that the azygous cerebral vein was present above the ventricles 187, but does not come into contact with the LV, and does not perfuse the CP tissue. As no large arteries were in direct contact with the lateral ventricles, the contribution of direct large vessel exchange detected at the long TE in the lateral ventricles can be assumed to be minimal, indicating therefore that the long TE ASL signal at the caudal aspect of the lateral ventricles predominantly reflects BCSFB-mediated transfer of water. However, as the TOF 3D FLASH dataset does not detect smaller arterioles, it is possible that direct exchange of labelled water from these vessels are still contributing to the appearance of signal.

Another factor which may contribute to possible “vessel contamination” is the appearance of intravascular spins from water within proximal vessels. Given the use of an ultra-long TE in BCSFB-ASL, the magnetization signal from water in the vessels will be nulled due to the markedly lower T2 of blood (30 ms @ 9.4T). In agreement with this, in both the higher resolution data used in this chapter (Figure 3 and Figure 4), and lower resolution data
acquired in other chapters, visual observation highlights the lack of vessel signal in the $M_C$ images and $\Delta M$ ASL images. When inspecting ASL images acquired at a lower in-plane and through-plane resolution (e.g. FOV: 20 mm $\times$ 20 mm, matrix: 32 $\times$ 32, slice thickness: 2.4 mm), we observed 2 distinct regions of signal at the lateral ventricles (2 $\times$ 6 voxels), which are used for manual ROI drawing. In higher resolution images, such as those obtained in this chapter, the ROI for analysis was drawn directly around the ventricular border. In both cases, the vessel identified from the FLASH dataset would be avoided in the ROI drawing.

In the human brain, a compartmental adaptation of the Buxton kinetic model was used to eliminate the contribution of vessel-localised spins. In the work presented in this thesis and by Evans et al. $^1$, the use of an ultra-long TE eliminates intravascular signals, and the adaptation of the 2-compartment model more accurately models what is being measured by BCSFB-ASL, i.e. by excluding the vascular compartment. Although the vessels identified from the angiography images are: i) not in direct contact with the ventricles, ii) not visible in the $M_C$ or $\Delta M$ images, and iii) not included in the ROI drawn for ASL, the adapted 2-compartment modelling provides further evidence that the quantified water delivery rates are not confounded by intravascular spins from large vessels, in the lateral ventricles of the mouse brain. Future work should take on a histological approach to determine colocalization of vessels of varying sizes around the lateral ventricles, which would further supplement the TOF 3D data by excluding the contribution of smaller arterioles to direct vessel exchange.

**Rat brain multi-TI – subarachnoid space**

T2-weighted anatomical MR data from the mouse brain that we have obtained to date has highlighted the small volume of the SAS in the mouse brain relative to the human brain. To overcome this issue, we chose to apply BCSFB-ASL in the rat brain where we were able to identify a prominent SAS proximal to the rostral cortex. Higher resolution multi-TI data were used to fit a 3-parameter Buxton kinetic model to extract values of bolus arrival time (tissue transit time, $\Delta t$), temporal length of the tagged bolus ($\tau$), and rate of labelled water delivery. A 3-parameter fitting approach extracts $\tau$ at a lower degree of precision. However, we opted
to fit for \( \tau \) rather than having a fixed value, to prevent assumptions of \( \tau \) values confounding the fitting of \( \Delta t \) and water delivery rates.

The pilot data revealed that compared to a BCSFB-ASL data obtained from a representative caudal LV region in a rat brain, the SAS (rostral cortex) had distinct water delivery kinetics; \( \Delta t \) and \( \tau \) were markedly longer at the SAS. Although promising in the pilot subject, extending this study to include a further 3 subjects was unsuccessful due to having poor quality fits (from visual observation, average fit Figure 8a and representative subject Figure 8c), thereby producing unreliable values of \( \Delta t \), \( \tau \), and rate of labelled water delivery. At this stage therefore, it is not possible to elucidate non-BCSFB mediated routes of delivery, as we are unable to reliably characterise the kinetics of the signal in this region.

To begin troubleshooting, we assessed the \( M_c \) data from these 3 subjects when fit to an IR curve. This analysis indicated that the average T1 value and inversion efficiency were in accordance with other CSF spaces investigated in this work. This suggests that the data is affected by noise in the \( \Delta M \) (ASL) data, as opposed to a systematic confound within the data acquisition protocol.

A key assumption of the Buxton modelling is that the \( \Delta M/M_0 \) will be zero prior to the arrival time of the tagged bolus to the voxel (\( \Delta t \)), and then will subsequently increase as a result of labelled water delivery, and then will reach a maximum. The group-averaged data displays two maxima – one at either side of \( T1 = 2.7 \) – which prevents the successful extraction of \( \Delta t \) (as well as \( \tau \) and flow) from the averaged data. We speculate that the appearance of an earlier peak reflects a high degree of noise in \( \Delta M \) values acquired at lower inflow times. Given the higher noise at early T1 values, we analysed the multi-T1 data, excluding \( \Delta M/M_0 \) values at T1 values below 1.50 s. This post-hoc explorative analysis produced a kinetic curve which was more in line with the pilot data, which displayed delayed arrival times relative to a representative rat caudal LV dataset (Figure 8b), i.e., 2.5 s compared to 1.12 s in the representative caudal LV dataset. This suggests that the long TE-ASL measurement employed here may be able to detect labelled water delivery to CSF spaces beyond the LV. Mechanistically, there may be non-CP mediated delivery routes with distinct kinetics at the SAS of the rat brain, such as AQP-1 mediated delivery of labelled arterial water across pial vessels at the SAS. Alternatively, the observation of an increased arrival time of the labelled
bolus may reflect the delayed arrival of labelled water as a result of initial delivery to the ventricular CSF via the BCSFB, and subsequent CSF-mediated transport to SAS regions.

Improvements to the data acquisition protocol appear necessary in order to reliably capture a putative long-TE ASL signal in the subarachnoid space in the rat brain. Future imaging paradigms should be designed to increase the sensitivity to differences at earlier TIs, which will aid comparisons of Δt. Obtaining paired data (caudal LV vs SAS signal) within each subject would allow for more informative statistical comparisons to be made. Lastly, a contributing issue in this experiment may be the investigation of a SAS region which is substantially smaller than the LV region. We used a rat model with the aim of locating a larger SAS region than in the mouse brain. However, SAS regions within the rat brain were also found to occupy a smaller portion of the brain. This small SAS region may not provide enough signal. Investigating the SAS at the cisterna magna may be more promising due to the increased volume of the CSF in this region.

Angiography data was collected in the mouse brain. In the study described here, ultra-long TE ASL data was collected from the SAS within the rat brain, as SAS regions are much smaller within the mouse brain. It is important to consider potential differences in the vasculature between the two rodent species. Previous work in rodents has highlighted that there are substantial similarities in the vasculature. However, making direct comparisons is particularly difficult as mapping of both arteries and veins in the whole mouse brain within a single study in mice has been done only recently. Furthermore, a key difference is that in the mouse brain, the circle of Willis at the base of the brain - an anastomotic collection of major vessels – has a more limited and variable capability to compensate for imbalances between both arterial supply systems. Lastly, comparison of BBB permeability in mice and rats revealed that brain capillary permeabilities are similar between the two species.

**Mouse brain multi-TI - CP-rich vs deficient regions**

To further characterise the sources of the BCSFB-ASL signal in CP-deficient regions, we compared BCSFB functional signals from regions which are either rich or deficient in choroid plexus tissue within the mouse brain ventricles. An increased arrival time in CP-deficient regions, relative to their CP-rich counterparts, would be indicative of distinct kinetics of the BCSFB-ASL signal at these regions. As the ventricular system is comprised of interconnecting
cavities, the distinct kinetics and appearance of signal in CP-deficient regions may be explained by a delay in arrival of labelled water from CP-rich regions due to CSF motion. Studies utilising invasive microsphere-based methods would often pool samples of choroid plexus tissue from both the left LV and right LV for autoradiography measurements. This pooling was not only required for smaller non-rodent mammal work, but was also conducted in in monkeys and dogs, where it may be considered more feasible to obtain more regional measurements of perfusion due to the larger tissue volumes. Thus, the impracticality of obtaining regional measurements has hindered studies of region-specific perfusion in the CP-ventricular system in animal models. The work presented in this chapter presents the first measurements describing differences in labelled water delivery in CP-rich vs deficient regions within the in-vivo rodent brain.

Firstly, the analysis highlighted that CP-deficient regions (rostral LV, bottom 3V) had significantly lower BCSFB-mediated flow rates of labelled water relative to their CP-rich counterparts (caudal LV, top 3V) (Figure 9). A reduction in flow here may simply reflect a difference in CP tissue distributions (Figure 1), which was also observed through single-TI measurements obtained for the purposes of signal validation using a single-TI approach, for example by Evans et al. (see Chapter 1, Figure 5b), as well in this work (see Chapter 3, Figure 11). Flow values in CP-deficient regions were non-zero. This observation may be a result of BCSFB-mediated water delivery across the CP tissue residing in the rostral end, i.e. there is less CP tissue in this region, but not none. The Allen mouse brain atlas (Figure 2, 106) suggests that the rostral LV and bottom 3V is devoid of CP tissue. This data originates from ex-vivo, histological analyses, where the choroid plexus is not in its native, hydrated state in-vivo. However, it is now understood that the in-vivo choroid plexus tissue pervades the ventricular system, analogous to a fishing net in the water. As such, it is possible that the CP tissue is extending out to regions believed to be deficient in CP, based on histological analysis. Similarly, in the human brain (where Petitclerc et al. applied an ultra-long TE ASL approach), micro-video probes inserted directly into the lateral ventricles reveal that the live choroid plexus expands to fill nearly all the cerebral ventricle. Thus, it is possible that in-vivo CP tissue within the
CSF-filled ventricles of the mouse brain may also expand to fill regions within the ventricular system that were previously thought to be CP-deficient.

There may also be rapid mixing from the caudal LV and top 3\textsuperscript{rd} ventricle into the rostral LV and bottom 3\textsuperscript{rd} ventricle, respectively. In addition, it may reflect delivery of labelled water to the CSF via other smaller vessels where the size and/or flow velocity were below the threshold require for clear visualisation from the angiography images (see Figure 6). At this stage, it is not possible to distinguish between these mechanisms.

Despite being sensitive to differences in flow, we were unable to detect differences in labelled water arrival times; \(\Delta t\) was not significantly different between CP-rich and their deficient counterparts. After removing datasets where \(\Delta t\) was less than a zero value (physiologically impossible), the data only bordered statistical significance. At this stage, the lack of statistical significance here is unlikely to be resolved by increasing our cohort size, but rather is likely to stem from the poor-quality individual subject fits of the multi-TI BCSFB-ASL data in CP-deficient regions (data not shown). Compounding with this issue, data at early TI values are also impacted by increased noise (assessed as the standard deviation of the ASL signal across the repetitions), rather than a genuine signal offset from the baseline, which therefore limits the precision at which we can detect \(\Delta t\). To explore this further, we conducted post-hoc statistical comparisons between the ASL (\(\Delta M/M_0\)) data from each subject and the theoretical zero value at the early inflow times prior to the arrival time where the labelled bolus has not arrived in the imaging voxels, i.e. at times \(<0.63\) s based on the caudal LV \(\Delta t\) observed in previous chapters and by Evans et al.\textsuperscript{1} (e.g. [Chapter 1, Figure 5a] and [Chapter 3, Figure 11]).

Here, 2-tailed, unpaired t-tests indicated that at TI = 0.2 s, the data was not significantly different from the zero baseline for all ventricular regions (\(p > 0.6\)). For TI = 0.75 s, which is beyond the expected \(\Delta t\) for the caudal LV, the \(\Delta M/M_0\) values were significantly greater than a zero baseline (\(p = 0.005\), i.e. reflects inflow of the tagged bolus after the arrival time. This was not shown to be significant for other ventricular regions (\(p > 0.3\)), therefore suggesting that arrival times may be longer for these regions, and that we are not sensitive to detecting \(\Delta t\) for these regions. However, further studies are required to affirm this post-hoc observation. Ultimately, this indicates that the imaging paradigm utilised in this study is not sensitive enough to detect differences in \(\Delta t\) due to the random noise in \(\Delta M/M_0\) values at early
TIs. We do not know the reason for greater ASL signal variability at the shorter TIs but this is something we are working to better understand.

**CSF motion and rapid mixing**

To investigate the possibility of CSF motion and rapid mixing contributing to the appearance of long TE ASL signal in CP-deficient regions such as the rostral LV and bottom 3rd ventricle, we assessed CSF motion semi-quantitatively. The multi-TI BCSFB-ASL approach was adapted: labelling was applied across a fixed, narrow imaging slice (1.00 mm) placed at the rostral end of the lateral ventricles, with three separate inversion slab thicknesses (1.05, 1.50, 2.00 mm). In these experiments, the interpretation of ΔM is distinct to ASL studies measuring perfusion. Here, ΔM represents the extent of dilution of the labelled water magnetization from within the imaging slice, as a result of the entry of spins (i.e. CSF, water) from outside the imaging slice. Therefore, by increasing the inversion slab thickness, and keeping a constant imaging slice thickness, we can detect the influence of inflowing CSF, as a result of rapid, back-and-forth CSF motion and mixing, as we move further away from the inversion slab.

Initial, qualitative assessment of the data suggests that at timescales similar to that of the typical BCSFB-ASL multi-TI acquisition, there is a marked contribution of inflowing spins from water molecules which arrive from outside the imaging slices in the BCSFB-ASL data, such as inflowing spins from CSF produced at the caudal end of the LV. As expected, we observe a decrease in ΔM/M0 as inversion slab thicknesses increases, alongside the increased delay in arrival (Δt) of the labelled water as we broaden our inversion slab. Together, the reduced ASL signal and increased arrival time, as inversion slab thickness is increased, reflects the increased distance that labelled water must travel prior to appearing in the imaging slice.

BCSFB-ASL multi-TI data obtained both within this chapter and previously1 indicate that ASL ΔM/M0 values are generally also in the order of 0.01-0.02 for BCSFB-mediated labelled water delivery at the caudal LV (Figure 9a). Rostral LV ASL signals were shown to be approx. 50% of caudal LV ΔM/M0 values (Figure 9a). Given that ΔM/M0 values are markedly higher in the CSF motion experiments described here, this suggest that CSF mixing may be making a significant contribution to our previous interpretations of BCSFB-ASL signals taken from CSF-rich vs deficient regions (Figure 9).
Figure 11 attempts to describe the effects of CSF mixing on measured magnetisation (M) and the ASL signal (ΔM) using different labelling schemes within this study. In our control image (globally inverted), the labelling slab will invert the magnetisation of all CSF across the body. However, in the labelling scheme used here (Figure 11a), at narrower inversion slab thicknesses there is a greater proportion of ventricular CSF adjacent to the imaging slice that does not have inverted magnetisation, and therefore will retain a high M_i value in the slice selective, labelled image (Figure 11b). Upon mixing with CSF that has been inverted within the imaging slice (low M_i) during the global inversion, this will yield an increased M_i within the imaging slice (Figure 11c) and a subsequently higher ΔM value upon subtraction (Figure 11d). As inversion slab thickness is increased here, a larger region of CSF adjacent to the imaging slice is inverted, and so there is a lower degree of mixing contributions around our imaging slice arising from unlabelled CSF. As a result, M_i in the imaging slice is increased to a lesser extent, so ΔM will not increase to the same degree as the use of narrower inversion bands.

In the optimisation of BCSFB-ASL in Chapter 3 and the initial validation of the approach [Evans et al.], by using a 20mm inversion slab, CSF is inverted identically in the global and slice selective images. Therefore, in our BCSFB-ASL measurements to assess water delivery in the caudal LV, CSF motion contributions are assumed to be minimal, as CSF within our imaging slice and CSF outside the imaging slice will both have similar inverted magnetisation values, yielding low ΔM contributions as a result. Therefore, our ΔM signal in the caudal LV will reflect the new arrival of labelled water into ventricular CSF across the CP tissue.
**Global control**  
Labelling scheme

**Labelled 1.05mm**

**Labelled 2.00mm**

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**Initial M**

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**M after CSF mixing**

CP-mediated labelled water delivery would decrease $M_L$ → increase $\Delta M$. Rapid CSF motion leads to dilution of inverted CSF water with adjacent unlabelled CSF → greater decrease in $M_L$ → greater increase in $\Delta M$.

$TE = 220$ ms

$M_L$ TI = 4 s

More unlabelled inflowing spins from adjacent CSF volume. $M_L$ increases more. $M_L \gg M_C$ $\Delta M$ larger.

$M_C$ TI = 4 s

Some unlabelled inflowing spins from smaller adjacent CSF volume. $M_L$ increases slightly. $M_L \gg M_C$ $\Delta M$ not as large.

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**ASL $\Delta M$**  
$TE = 220$ ms

$\Delta M$ TI = 4 s
**Figure 11 – CSF motion and rapid mixing: effects on magnetisation.** Schematic showing the effects of CSF mixing on the detected control/label magnetisation and ΔM with ultra-long TE ASL.

a) Various labelling schemes: non-selective/global/control scheme, as well as the slice-selective labelling schemes (1.05mm and 2.00mm used in these experiments). Sagittal orientation.

b) Expected magnetisation distribution following inversion pulse application. Darker regions correspond to areas where water molecules have had their magnetisation inverted. Sagittal orientation.

c) Top row: expected magnetisation distribution localised to the ventricular CSF system (sagittal orientation), following CSF mixing (red arrows).
Bottom row: $M_C$ and $M_L$ data from CSF mixing experiments (coronal orientation, averaged image at TI = 4000 ms).

d) Top row: expected ΔM distribution localised to the ventricular CSF system (sagittal orientation), following CSF mixing.
Bottom row: ΔM data from CSF mixing experiments (coronal orientation, averaged image at TI = 4000 ms).

In Chapter 3, ASL measurements were obtained in a water phantom where inversion slab thickness was optimised in order to determine the inversion width required to provide a value of zero blood flow in the phantom. This data suggested that the use of narrow inversion slab thicknesses (e.g. < 2.00 mm) may give rise to potential slice profile and subtraction artefacts (see Chapter 3, Figure 2). RF pulse effects on the ASL signal are predicted to be similar between CSF in the mouse brain and a water-filled phantom, and as such, any deviation of the ASL signal here can be attributed to the effects of CSF mixing. In this work, we are able to detect changes primarily reflective of the effects of rapid CSF motion. Firstly, in this work, the ΔM/M0 data as a function of TI gradually increases and then, after a peak, decreases, thereby suggesting that the effects observed here are not caused by artefacts, but instead are driven...
primarily by a physiological factor, namely CSF motion. In addition, the $\Delta M/M_0$ values using a narrow inversion slice in this study are an order of magnitude greater than the values obtained from a water phantom ($\Delta M/M_0 \sim 0.01$ in water phantom, vs $\sim 0.1$ here). This highlights that, similar to ASL signals derived from BCSFB-mediated water delivery at the caudal LV, slice profile artefacts lead to changes in $\Delta M/M_0$ which are of a much smaller magnitude than the effects of CSF motion we are sensitive to in these in-vivo measurement. Ultimately this data indicates that, when attempting to characterise brain-wide BCSFB-ASL signals, there will be a contribution from mixing of spins from CSF compartments, as well as differences in CP-tissue content. As such, for the interpretation of data obtained in CP-rich vs deficient regions in the mouse brain, the non-zero perfusion values obtained in CP-deficient regions, and differences in BCSFB-ASL kinetics will not only be influenced by a difference in CP tissue density, but will also be influenced by the inflow of spins from rapid back-and-forth CSF mixing. However, as the analysis of this data is semi-quantitative, much like the use of a TimeSLIP approach $^{184}$, it is difficult to quantify the contribution from CSF rapid motion and mixing to the appearance of long-TE ASL signals in CSF regions beyond the caudal aspect of the lateral ventricles.

To supplement this work, there is potential for the use of phase contrast MRI to quantitatively map whole-brain CSF flow $^{193}$. Although difficult in rodents due to the low CSF velocities relative to humans, very recent work by Li et al. has demonstrated the feasibility of obtaining measurements of CSF flow ($\sim 0.2$ m/s under isoflurane) in mice using a novel phase contrast approach $^{194}$. It would be advantageous in future studies to apply an axial orientation for the BCSFB-ASL EPI readout which includes multiple regions of interest, such as the SAS at the rostral cortex, as well as several ventricular regions (rostral and caudal LVs, top 3V).

**Conclusion**

In this chapter, we worked towards further characterisation of the novel BCSFB-ASL signal, particularly in CSF spaces beyond the caudal LV. Pilot data in the SAS of the rat brain revealed the possibility of being able to extract reliable water delivery values with distinct kinetics. However, data from the larger cohort indicated the requirement for further optimisation of the imaging paradigm and identifying a more prominent SAS region to study. Comparing CSF
regions within the mouse ventricles indicated that CP-deficient regions had reduced but non-zero water delivery values. However, at this stage, we were unable to distinguish whether this reflect alternate routes of entry of labelled blood water into the CSF space (i.e. non BCSFB derived) or if this is a result of rapid, back-and-forth CSF mixing effects.
5. Chapter 5 – Investigating changes in blood-cerebrospinal fluid barrier function in a rat model of chronic hypertension using non-invasive MRI

a. Introduction

The latest figures provided by the World Health Organisation estimate that 1.28 billion adults suffer from hypertension. Hypertension is an established risk factor for the onset of neurodegenerative conditions such as Alzheimer’s Disease (AD). However, there is a limited understanding of the complex changes occurring in the brain under systemic hypertension that lead to downstream neurodegeneration. Moreover, as well as building understanding, it is important to also work towards development of biomarkers for the early detection of brain pathology as a consequence of sustained hypertension.

Disruption of the cerebral vasculature has been well established to originate from the effects of hypertension, e.g. impairment of blood brain barrier (BBB) integrity and reductions in cerebral blood flow (CBF). However, the responsibility of mediating the complex interplay between blood and the brain, a key mechanism for central nervous system (CNS) homeostasis, is not limited to only the role of the BBB alone. This responsibility is also attributed to the blood-cerebrospinal fluid barrier (BCSFB) at the choroid plexus (CP) – a relatively understudied barrier within the brain’s cerebrospinal fluid-filled ventricles which display a selective vulnerability towards hypertension-driven damage. Prior evidence points to the extent of hypertension-induced damage being more pronounced at the BCSFB than the BBB, which manifests as increased BCSFB leakiness and an altered cerebrospinal fluid (CSF) homeostasis in a manner akin to that observed in AD. The limited study of BCSFB functionality in-vivo, due to the reliance on invasive methodologies, has hindered exploration of pathophysiological changes at the BCSFB that may catalyse neurodegenerative processes, particularly under hypertension.

We have recently developed a translational MRI technique for the non-invasive assessment of BCSFB function, known as ‘BCSFB Arterial Spin Labelling (ASL)’. BCSFB-ASL quantifies the
rate of BCSFB-mediated delivery of endogenous arterial blood water to ventricular CSF, thereby providing a surrogate measure of BCSFB function \(^1,\)\(^{201}\). This non-invasive approach may help to better elucidate the BCSFB’s role in the onset of downstream neurodegeneration in the hypertensive brain. Here we present the application of this technique to probe BCSFB functional changes under systemic hypertension, as well as demonstrating the first application in the rat brain.

Spontaneous hypertensive rats (SHR) provide a well-characterised model of human hypertension and present with markers of neurodegeneration such as amyloid-β accumulation, cognitive impairment, cerebral atrophy, BBB dysfunction, and brain fluid dysregulation \(^{202–206}\). Using invasive methods, previous studies have detected functional irregularities at the BCSFB locus in the SHR model \(^{199,200,207}\). Employing a non-invasive standard-ASL and BCSFB-ASL approach, we investigated whether the impairment of BCSFB function is detectable using non-invasive MRI techniques in the SHR model, relative to Wistar Kyoto (WKY) controls. Additionally, we quantified changes in cortical CBF - a conventional biomarker for cerebrovascular health \(^{208}\) - as well as obtaining measures of T1\(_{\text{CSF}}\) and ventricular volumes to assess CSF homeostasis. We hypothesise that non-invasive measures of BCSFB function will reveal its derangement under systemic hypertension.

The work presented in this chapter is an adaptation of published research (see Inclusion of Published Work (2)). Supplementing this published work, this chapter also describes post-hoc analysis conducted towards the aim of addressing deviations in the BCSFB-ASL multi-TI signal from the adapted kinetic modelling.

**b. Methods**

**Data collection**

All imaging data within this chapter were collected by me. Invasive blood pressure measurements in animals were conducted by Alla Korsak (Centre for Cardiovascular and Metabolic Neuroscience, Neuroscience, Physiology and Pharmacology, University College London, London, United Kingdom).

**Animal preparation**
All animal procedures were performed under the UK Home Office Act (Scientific Procedures, 1986). SHR and WKY normotensive rats (provided by Envigo) were used in our study (male, n = 6 in each group). A pilot study for the optimisation of parameters was conducted using the WKY subjects at 10 weeks old. Following parameter optimisation, the final data collection was conducted with SHRs and WKYs at 21 weeks old. Body weight measurements at the time of scanning were: WKY 348 ± 5.0 g vs SHR 369 ± 9.5 g.

Prior to commencing MRI acquisitions, subjects underwent anaesthetic induction using 4% isoflurane in 0.8 L/min medical air and 0.2 L/min O₂. Following induction and weighing, rats were placed into the MRI cradle with bite bar, nose cone and ear bars to ensure a well secured position of the rat head to minimise motion during the data acquisition. Eye ointment was also applied to prevent drying.

A scavenger pump was placed inside the magnet bore to prevent isoflurane build-up. Anaesthesia was maintained during the acquisition by reducing isoflurane concentration to 2% in 0.4 L/min room air and 0.1 L/min O₂.

Temperature and breathing rate were monitored throughout all the experiments using a rectal probe and a respiration pad (SA Instruments). Rat temperature was maintained at 37 ± 0.5 °C using heated water tubing during data acquisition.

**Magnetic resonance imaging (MRI) protocols**

MRI protocols were applied to inform on BCSFB function, CSF homeostasis, and cerebrovascular health (Figure 1). Images were acquired on a 9.4 T Bruker imaging system (BioSpec 94/20 USR) with a horizontal bore and 440 mT/m gradient set with an outer/inner diameter of 205 mm /116 mm respectively (BioSpec B-GA 1252), 86 mm volume coil and a four-channel array rat brain surface coil (RAPID Biomedical GmbH) for the transmission and the reception of the RF signal, respectively. The centre of the volume coil was positioned caudally 3 cm from the iso-centre of the magnet to ensure a good labelling efficiency of the global inversion pulse during the flow-alternating inversion recovery (FAIR)-ASL acquisition.
Figure 1 – MRI protocols: non-invasive multi-inflow time (multi-TI) arterial spin labelling (ASL) and structural, T2-weighted MRI to inform on hypertension-induced impairment of BCSFB function, CSF homeostasis and cerebrovascular health (created with Biorender.com).

Anatomical reference scans

A T2-TurboRARE sequence (fast-spin echo, Paravision v6.0.1) was used to collect sagittal and coronal anatomical reference images to clearly visualise the location of the major CSF compartments in the rat brain. Sequence parameters were: (field of view (FOV) = 30 mm × 30 mm; matrix size = 256 × 256; RARE factor = 8; effective echo time (TE) = 33 ms; repetition time (TR) = 2500 ms.

Sagittal anatomical reference images (12 × 1 mm slices) were used to position the axial anatomical reference imaging slice and the ASL imaging slices. Coronal anatomical reference images (12 × 0.4 mm slices, 4.8 mm total) were manually positioned to align with the caudal
region of the lateral ventricles. These coronal images were subsequently manually segmented to provide an estimate of lateral ventricular volume for each of the subjects.

**Arterial Spin Labelling MRI Protocols**

Each ASL data set was acquired using a flow-sensitive alternating inversion recovery (FAIR) sequence with a single slice, single shot spin echo – echo planar imaging readout, 20 mm slice-selective width, and a global labelling pulse, across all the experiments.

Parameters for standard-ASL: 2.4 mm slice thickness, matrix size = 32 × 32, FOV = 32 mm × 32 mm, 4 dummy scans, TE = 20 ms. Inflow times (TI) = [200, 500, 1000, 1500, 2000, 3000, 4000, 6000 ms], using TR = 12000 ms, 5 repetitions per TI.

Parameters for BCSFB-ASL: single slice, 4.8 mm slice thickness, matrix size = 32 × 32, FOV = 32 mm × 32 mm, 6 dummy scans, TE = 220 ms. TI = [200, 750, 1500, 2750, 4000, 5000, 6000 ms], using recovery time = 12000 ms, 10 repetitions per TI.

Importantly, as described in Chapter 1 - Section I, the ASL imaging slice was manually positioned to align with the caudal end of the lateral ventricles, as it has been previously shown to be the predominant region within the lateral ventricles occupied by the CP. The large slice thickness was chosen to ensure that the slice contained the majority of the lateral ventricles (excluding the more rostral sections which are known to not contain choroid plexus tissue). Therefore, as described in our recent work, measurements of BCSFB function are concentrated to CP within the lateral ventricles and not the 3rd and 4th ventricles.

**MRI data analysis**

In order to obtain absolute, quantified values of cortical CBF (standard-ASL, TE = 20 ms) and BCSFB-mediated water delivery (BCSFB-ASL, TE = 220 ms) from multi-TI data, we followed the analytical pipeline described in Chapter 1 (Section I, Figure 8 and Figure 9) and Chapter 3 (Section b).

For the BCSFB-ASL technique, our approach was to take the sum of the BCSFB - ASL signal in the lateral ventricles (Figure 2). This would enable the measurement of the total amount of labelled arterial-blood-water delivery to ventricular CSF, thereby yielding an overall measure...
of total BSCFB function in the lateral ventricles. Previous multi-TI data acquired in the mouse brain utilised an ROI volume of 11.25 mm$^3$. Given the larger volume of the lateral ventricles in the SHR brain, the BCSFB-ASL image analysis process was adapted so that a larger ROI could be overlaid with the position of the lateral ventricles on the slice-selective ASL image: two 3 × 3 voxel ROIs, 18 voxels in total, ROI volume = 86.4 mm$^3$ $^{201}$. 

The outputs of the model fittings provided subject-wise quantitative values for cortical perfusion (standard-ASL images at TE = 20 ms), and rates of BCSFB-mediated water delivery (BCSFB-ASL images at TE = 220 ms). We report the group average of the individually extracted values of BCSFB-mediated water delivery rate, cortical CBF and T1$_{CSF}$ across the lateral ventricles.

Taking the average rate of BCSFB-mediated labelled water delivery to the lateral ventricles and incorporating the total size of the functional voxels (86.4 mm$^3$) returns a total BCSFB-labelled water delivery rate to the lateral ventricles.

For the purpose of visual comparison of the group-averaged kinetic curves (e.g. Figure 5b,d), we averaged the values of ΔM/M0 for standard-ASL, or ΔM/M0$_{corr}$ for BCSFB-ASL.
Figure 2 - ROI selection for BCSFB-ASL images. Left: examples of WKY (A) and SHR (B) ROIs (green) are shown on control (*M*, non-selective FAIR) images (*TI = 4 s, n = 1 per group*). Right: examples of T2-weighted coronal anatomical images for reference (*n = 1 per group*).

**Pilot study for parameter optimisation**

The pilot study in 10-week-old WKY controls (*n = 6*) were used to fit a 2-compartment Buxton Kinetic model to extract 3 variables: the rate of labelled water delivery, as well as arrival time (*Δt*) and temporal length (*τ*) of the tagged bolus of arterial blood water. Based on this pilot dataset, the arrival time (*Δt = 1.04 s*) and temporal length (*τ = 3.66 s*) values were fixed when fitting the 2-compartment Buxton kinetic model to datasets acquired at 21-weeks, in order to extract solely the rate of arterial water delivery with a higher degree of precision (Figure 3).
**Measurements of blood pressure**

Following completion of the MRI protocols, confirmation of the subjects’ hypertensive state was confirmed through invasive blood pressure measurements in 4 of the subjects within each group (8 in total). Here, an identical anaesthesia protocol as used for MRI was employed. Arterial blood pressure was measured through an arterial catheter in the femoral artery and connected to a physiological monitoring system. Data were sampled at 400 Hz for the recording of arterial blood pressure for a baseline reading over 20 minutes.

**Statistical methods**

Statistical comparisons between SHR and WKY data were made using unpaired, 2-tailed, Student’s t-tests (GraphPad Prism 9 and Microsoft Excel).

**c. Results**

**Pilot study**

A pilot study was conducted in 10-week-old WKY subjects (n = 6) to extract initial modelling parameters. Application of a 2-compartment Buxton kinetic model yielded an average total water delivery rate of 13.3 ± 3.7 μl/min, as well as arrival time (Δt = 1.04 s) and temporal length (τ = 3.66 s) of the tagged bolus (Figure 3).
**Figure 3 – Pilot data: averaged BCSFB-ASL multi-TI data** (WKY, 10-weeks old, n = 6). Error bars: ±SEM. GOF, $R^2 = 0.9301$.

**BCSFB-mediated water delivery**

Recent evidence indicates that the CP-BCSFB system is notably perturbed by systemic hypertension 199,200, as such we applied a multi-TI BCSFB-ASL MRI approach in SHR and WKY subjects (n = 6 in each group, 21-weeks old) to measure the degree a change in BCSFB derangement under hypertension (Figure 1).

The total rate of BCSFB-mediated labelled arterial water delivery to ventricular CSF was extracted from multi-TI BCSFB-ASL data (Figure 4, Figure 5a,b, ). We measured a 35.8 %, decrease in the total rate of water delivery in the SHRs compared to WKYs, which can be observed in the extracted water delivery values WKY 14.4 ± 1.92 μl/min vs SHR 9.22 ± 1.20 μl/min (p = 0.037, 2 -tailed unpaired t-test, Figure 5a), as well as from visual inspection of the group-averaged BCSFB-ASL kinetic curves (Figure 5b).
Figure 4 - BCSFB-ASL multi-TI data: individual subject fits to Buxton kinetic model. (A) WKY cohort (n = 6). (B) SHR cohort (n = 6). GOF (R²) values are displayed alongside each fit. Error bars: ± standard deviation across 10 repetitions at each TI.
Cortical CBF

Changes in CBF under chronic hypertension can inform on cerebrovascular health. However, CBF changes under hypertension have shown to be variable. Here, we applied a multi-TI standard-ASL approach for the extraction of cortical CBF (Figure 5d). Cortical CBF was not significantly different between the WKY normotensive controls and the SHRs, as shown by the group-averaged (n = 6) fitted perfusion values: WKY 123 ± 7.4 ml/min/100 g vs SHR 118 ± 8.7 ml/min/100 g (p = 0.70, Figure 5c). The group-averaged standard-ASL kinetic curves also demonstrate the strong similarity of the two groups (Figure 5d).

Figure 5 - Multi-TI kinetic data for BCSFB-ASL and Standard-ASL.

A) Extracted BCSFB-mediated total water delivery in WKY and SHR subjects.
B) Averaged BCSFB-ASL multi-TI data alongside kinetic curve fits for WKY and SHR subjects.
C) Extracted cortical CBF in WKY and SHR subjects.
D) Averaged standard-ASL multi-TI data alongside kinetic curve fits for WKY and SHR subjects.
Error bars: ±SEM (n = 6 WKY, 6 SHR). Asterisks: 2-tailed t-test significance, [* = (p < 0.05)], [** = (p < 0.01)], [*** = (p < 0.001)], [**** = (p < 0.0001)].

CSF homeostasis

Previous reports have shown that SHRs display marked changes in brain fluid management, as determined through measuring perturbations in CSF secretory profiles, glymphatic flow, brain water mobility and ventriculomegaly. To further probe how hypertension impacts CSF volume and composition, we acquired measures for surrogate markers of CSF management: T1_{CSF} and lateral ventricular volumes.

Single subject examples of data obtained from WKY and SHR groups of the signal (M_r) variation with T1 obtained using BCSFB-ASL, alongside the fits to the simple inversion recovery model is depicted in Figure 6b. T1_{CSF} was found to be significantly higher in the lateral ventricles of the hypertensive rats: SHR 4.58 ± 0.06 s vs WKY 4.24 ± 0.04 s (p = 0.0012, Figure 6a). Cortical tissue T1 was not significantly different between the two groups: SHR 1.91 ± 0.01 vs WKY 1.88 ± 0.01 s (p = 0.14, data not shown).

Volumes of the lateral ventricles were quantified for all subjects using T2-weighted anatomical images (Figure 6c,d). SHR subjects had a ~1.5-fold increase in their lateral ventricular volumes relative to normotensive controls: WKY 56 ± 3.0 mm^3 vs SHR 83 ± 1.0 mm^3 (p = 6 × 10^-6, Figure 6c). This is supported by visual inspection of the T2-weighted anatomical images (equivalent slices) from SHR and WKY subjects (Figure 6d).
Figure 6 - CSF homeostasis: $T_{1CSF}$ and lateral ventricular volume

A) $T_{1CSF}$ values from WKY and SHR subjects, extracted from BCSFB-ASL multi-TI data. Error bars: ±SEM (n = 6 WKY, 6 SHR).

B) Example of multi-TI inversion recovery fitting (n = 1 WKY, 1 SHR). Error bars: ±stdev (20 repetitions at each TI).

C) Lateral ventricular volume values from WKY and SHR subjects, from T2-weighted anatomical images. Error bars: ±SEM (n = 6 WKY, 6 SHR).

D) Examples of T2-weighted anatomical images (equivalent slices) displaying ventriculomegaly (n = 1 WKY, 1 SHR).

Asterisks: 2-tailed t-test significance, [* = (p < 0.05)], [** = (p < 0.01)], [*** = (p < 0.001)], [**** = (p < 0.0001)].
Blood pressure measurements

Measurement of blood pressure in our SHR (n = 4) and WKY (n = 4) rats confirmed their respective blood pressure states (Figure 7). SHRs were shown to have a significantly higher blood pressure compared to WKY controls, which was evident from systolic (SHR 185 ± 6.5 vs WKY 105 ± 12 mmHg, p = 0.0010), diastolic (SHR 113 ± 7.5 vs WKY 55 ± 7.4 mmHg, p = 0.0016) and mean blood pressure (SHR 149 ± 6.9 vs WKY 80 ± 9.5 mmHg, p = 0.0011). Additionally, SHRs were found to have an increased blood pressure range (systolic-diastolic) compared to WKYs [SHR 73 ± 2.5 vs WKY 50 ± 5.4 mmHg, p = 0.009).

![Figure 7 - Group-averaged arterial pressures from WKY and SHR subjects. Error bars: ±SEM (n = 4 WKY, 4 SHR). Asterisks: 2-tailed t-test significance, [* = (p < 0.05)], [** = (p < 0.01)], [*** = (p < 0.001)], [**** = (p < 0.0001)].](image)

Post-hoc exploration: bias in the BCSFB-ASL signal

Further, post-hoc analysis was conducted to determine whether there were systematic biases in the BCSFB-ASL signal. These offsets in the measurement which appear to be outside the range of estimated errors are present primarily in the early TI values (TI = 200 ms, 750 ms),
and particularly for the SHR cohort. Statistical comparisons were conducted between the ASL ($\Delta M/M_0_{\text{corr}}$) data from each subject and the theoretical zero value at the early inflow times, i.e. prior to the arrival time ($\Delta t = 1.04$ s) where the labelled bolus has not arrived in our imaging voxels. A 2-tailed, unpaired t-tests indicated that at TI = 200 ms and 750 ms, the data was not significantly different from the zero baseline (TI = 200 ms [WKY p = 0.26, SHR p = 0.13], TI = 750 ms [WKY p = 0.37, SHR p = 0.39]).

Furthermore, we compared the standard deviations (stdev) of our BCSFB-ASL $\Delta M/M_0_{\text{corr}}$ values across the range of TIs (Figure 8). From the 10 repetitions per TI acquired in each subject, a standard deviation value was calculated. Standard deviation values were then averaged for each cohort to provide average stdev. Our analysis indicates that the standard deviations at the lower inflow times, particularly for our SHR cohort, are much higher.

![Figure 8](image)

**Figure 8 – Standard deviation of $\Delta M/M_0_{\text{corr}}$ at different TI values.** Points indicate the averaged standard deviation (stdev) from the 6 subjects in each cohort. Error bars: ±SEM.

d. **Discussion**

Patients suffering from systemic hypertension face an increased risk of cognitive decline later in life. Thus, there is a need for not only an improvement in our understanding of disease aetiology, but also for early biomarkers of upstream pathological processes. Addressing the need for non-invasive methodologies, we recently developed BCSFB-ASL - a translatable ASL-MRI approach which provides a surrogate measure of BCSFB function by quantifying the rate
of labelled endogenous arterial blood-water delivery, mediated by the BCSFB, into ventricular CSF. In this work we record a 36 % reduction in the rate of BCSFB-mediated water delivery in the SHR model relative to normotensive controls, indicating an impaired BCSFB function under chronic hypertension. We also measure perturbations in CSF homeostasis, as measured by changes in $T_{1CSF}$ and lateral ventricular volume. However, the cortical CBF measurements revealed no changes under hypertension.

**Cortical CBF**

To date, the bulk of work investigating the mechanisms underpinning hypertension-driven neurodegeneration have been centred on studying the cerebral vasculature, revealing impairments to the integrity of the cellular network at the BBB and the associated abnormalities in CBF and cerebrovascular reactivity. These changes are thought to increase the brain’s vulnerability towards neurodegenerative diseases. However, despite serving as an established biomarker for neurovascular pathology, there are inconsistencies in the directionality of change for CBF in hypertension studies. Baseline CBF in SHRs has been shown to increase from elevated mean arterial blood pressures, but other studies point to CBF decreasing or remaining similar due to autoregulatory mechanisms. Our multi-TI conventional ASL data revealed no significant difference in the cortical CBF between hypertensive and normotensive groups, likely due to vasoconstrictive autoregulation previously reported in SHRs of a similar age. Our data, combined with the variability in the literature, suggests that CBF measurements may not be the most sensitive biomarker of functional derangement under hypertension.

**BCSFB-mediated water delivery**

The BCSFB-ASL methodology was developed and applied first in the mouse brain. Here, we present the application of BCSFB-ASL under systemic hypertension to investigate functional impairment, as well as the first application of this methodology in the rat brain. In this study, there is a good fit of a 2-compartment Buxton Kinetic model to the multi-TI data, and similar to the initial characterisation in the mouse brain, the BCSFB-ASL kinetic curve has features distinct to that of the standard-ASL dataset, i.e. a delayed arrival time ($\Delta t$) for the tagged bolus, delayed time to peak for the ASL signal, and a reduced rate of ASL-signal decay due to the lengthened $T1$ of lateral ventricular CSF relative to cortical tissue.
The healthy rat brain was found to have a total BCSFB-mediated water delivery rate of 14.4 µl/min, which, as expected, is markedly higher than the rate observed in the mouse brain (2.7 µl/min) \(^1\), given that the volume of the rat brain is \(\sim4.5\) times larger \(^{218,219}\). By providing an initial reference value for BCSFB-mediated water delivery to ventricular CSF in the healthy (WKY) and hypertensive (SHR) groups, we have demonstrated the feasibility of this measurement in larger animal models. In regards to clinical translation, recent work has captured the delivery of labelled blood water into the CSF in the human brain \(^{132}\) using ultra-long TE ASL, and other studies have applied traditional ASL methods to estimate apparent choroid plexus perfusion, with promising results \(^{131,220,221}\).

We detected a marked reduction in rates of BCSFB-mediated water delivery in the SHR providing evidence that the BCSFB-ASL technique can detect derangement of the CP-BCSFB locus in hypertension. The limited study of the CP-BCSFB system \textit{in-vivo} has been due to the lack of non-invasive methodologies and has historically necessitated the use of radioactive tracers or contrast agents alongside terminal surgical methods \(^{155,192}\). Given this limitation, the role of the BCSFB in hypertension has only been assessed in \textit{ex-vivo} brain tissues \(^{199,200}\). This literature points to the selective vulnerability of the BCSFB to hypertensive damage, with damage manifesting as a disruption in BCSFB integrity, and an alteration in the profile of proteins secreted into the CSF, which will affect CSF homeostasis and subsequent overall CNS function \(^{199,200}\). Furthermore, hypertensive BCSFB damage was reported to resemble the BCSFB impairment observed with Alzheimer’s Disease \(^{199}\), thus further implicating the BCSFB in the development of dementia. Together, these previous reports support the existence of marked hypertension induced BCSFB impairment. However, the invasive nature of these methodologies to determine BCSFB impairment has a limited capacity for studies of \textit{in-vivo} disease progression, which our translatable measures of BCSFB function show the potential to provide.

A plot study was conducted in 10-week-old WKYs to extract estimates for \(\Delta t\) and \(\tau\), which would be fixed in subsequent modelling. Visual inspection of the output model fittings indicates that the assumed value of \(\Delta t\) is similar in the 21-week and 10-week-old WKY cohorts. Although, there is limited confidence in the accuracy of this arrival time for the SHR cohort due to the noise present in the BCSFB-ASL signal at early inflow time values. Furthermore, CBF studies in grey matter have revealed that the arrival time of a labelled bolus of water is
dependent on subject age, i.e. an increase in arrival time with age \(^{222,223}\). Thus, \(\Delta t\) values extracted from ASL data from younger, normotensive subjects may not be reflective of \(\Delta t\) values in older subjects. However, from visual inspection of the individual and cohort-averaged kinetic fits (Figures 3, 4 and 5), it does not appear that \(\Delta t\) is substantially lengthened when comparing data from 10-week and 21-week-old WKY data.

Post-hoc analysis sought to determine whether systematic biases were present in the BCSFB-ASL signal at early inflow times, where deviations were seen from the expected \(\Delta M/M_0\text{corr} = 0\) value, prior to the arrival of the tagged bolus. This deviation from the modelled signal has also been observed previously within this thesis (e.g. Chapter 3: Figure 14. Comparison of a theoretical zero baseline to the individual subject \(\Delta M/M_0\text{corr}\) values using a t-test revealed no significant difference. Furthermore, investigation into the standard deviations of the BCSFB-ASL signal across the subjects at the selected TI values indicated that shorter TI values were subject to increased levels of variability (higher standard deviations), and were markedly higher for the SHR cohort. Taken together, this analysis ultimately indicates that any deviations from the model at the early TI values reflect random variability of the \(\Delta M/M_0\text{corr}\) data, and not a systematic signal offset from zero at these lower TIs. We do not know the reason for greater ASL signal variability at the short TIs, but is something we are working to better understand.

Previous work aiming to investigate how hypertension may modulate BCSFB permeability in the brain has been relatively limited. 42-week-old SHRs were found to display no change in the permeabilities of both the BBB and BCSFB when quantifying fluorescein leakage \(^{217}\). In contrast, measurements of barrier integrity using \(^{14}\)C-sucrose in 12–16-week-old SHRs revealed an increased permeability of the BCSFB \(^{200}\), with no difference in BBB permeability detected. The BCSFB-ASL signal, measured here, reflects the average rate of perfusion to the CP convolved with the permeability of the BCSFB to water (the ‘extraction fraction’ \(^{157}\)) and the mass of the CP tissue within the lateral ventricles. Therefore, considering the aforementioned report of increased BCSFB-permeability in the SHR brain, this may suggest that the decreased rates of BCSFB-mediated water delivery detected here primarily reflect decreased CP perfusion. It is important to note however, that changes in the permeability of the BCSFB to larger tracers may not be indicative of changes in permeability to water (where, for example, Aquaporin-1 is thought to play an important role). Despite the limited specificity
of the BCSFB-ASL measurement to changes in BCSFB physiology, it may still represent a valuable biomarker of BCSBF derangement given its non-invasive nature and good reproducibility \textsuperscript{1} to probe the function of this difficult-to-measure structure.

There is discord in the invasive studies assessing changes in CSF secretion rates in SHRs relative to normotensive controls. Al-Sarraf & Philip show that rates of CSF secretion are increased in 12-16 week-old SHRs, using the ventriculocisternal perfusion method (Masserman technique, Chapter 1 – Section f) \textsuperscript{93}. On the other hand, using a similar methodology, Naessens et al. found no significant difference in rates of CSF secretion in 42-week-old SHRs \textsuperscript{217}. This discrepancy may stem from differences in age between SHR cohorts studied, as well as the inherent difficulty in obtaining reliable ventriculocisternal perfusion measurements. In this work, we detect decreases in BCSFB-mediated water delivery. However, given that this is a measure of total, rather than net, labelled water delivery across the BCSFB into ventricular CSF, we are unable to draw a direct comparison to rates of CSF secretion, which describes the net transport of water and solutes (see Chapter 1 – Section h, Section i). Future experiments seeking to correlate invasive measures of CSF secretion with the BCSFB-ASL signal will determine the extent to which BCSFB-ASL measurements can serve as a correlate of CSF secretion rates. The SHR model exhibits a range of neurodegenerative changes that occur later in life for patients suffering from chronic hypertension: BBB dysfunction with high levels of amyloid-B accumulation \textsuperscript{206}, non-spatial memory deficits, brain atrophy, neuroinflammation, and dysregulated brain fluid (ISF and CSF) homeostasis \textsuperscript{205}.

Here, our sensitivity to BCSFB dysfunction suggests that obtaining such \textit{in-vivo} measures may be informative for early detection of the changes that occur in the hypertensive brain that may accelerate the progression of neurodegenerative pathways. Importantly, given the minimal changes in CBF observed in this work, investigation into BCSFB function may provide distinct staging of disease processes relative to more conventional biomarkers of cerebrovascular health. Future studies could perform longitudinal measures of BCSFB function together with imaging biomarkers of neurodegeneration to characterise the timeline and possible predictive relationship of such measurements.

\textbf{CSF homeostasis}

The BCSFB-ASL multi-TI approach also permitted an estimation of changes in T1\textsubscript{CSF} under hypertension, which may arise from alterations in the secretory profile of signalling factors by
the CP-BCSFB system that occur concurrently with structural and functional barrier decline\textsuperscript{199}. The changes in CSF management are indicative of brain fluid dysregulation under hypertension which are in line with previous imaging studies, e.g. through abnormalities of glymphatic CSF transport measured with dynamic contrast-enhanced-MRI (DCE-MRI) using the infusion of a gadolinium-based contrast agent into the cisterna magna, in both young (~8 week-old) and adult (~20 week-old) SHRs\textsuperscript{205}. Imbalance of brain fluid management has also been observed through a reduction in apparent diffusion coefficient (ADC) values in 45-week-old SHRs\textsuperscript{202}. By measuring BCSFB function alongside T1\textsubscript{CSF} changes, this provides the potential for an increased sensitivity to changes in the early phases of hypertension-driven neurodegeneration in a manner that is more translatable than contrast agent infusion into the cisterna magna for DCE-MRI studies of glymphatic function.

Changes in T1\textsubscript{CSF} may stem directly from impairments in BCSFB function in the hypertensive cohort, such as alterations in the secretion of proteins into the CSF. For example, transferrin (Tf), the major iron transport protein in the brain, is secreted by the choroid plexus into the interstitial fluid (ISF) to acquire Fe\textsuperscript{3+}, which will then equilibrate with the CSF\textsuperscript{224,225}. Relaxivity data obtained from blood serum has shown that Tf and other proteins such as globulins and albumin contribute to the serum T1 relaxation times\textsuperscript{226}. It is likely that the changes in CSF protein composition in hypertensive rats will influence the T1\textsubscript{CSF} values, i.e. potential reductions in CSF Tf-Fe\textsuperscript{3+} levels would lengthen the measured T1\textsubscript{CSF}.

Ventriculomegaly in the hypertensive subjects in this work is similar to the increases in volume measured previously in rats of a similar age\textsuperscript{202}, as well as in hypertensive patients\textsuperscript{227–229}. The expansion of this region is reported to arise from a combination of factors including: increase in CSF production rates\textsuperscript{200}; increased CSF reabsorption resistance; or ventricular expansion due to cerebral atrophy\textsuperscript{217}. Given that ventriculomegaly is a hallmark of systemic hypertension, this provides an opportunity to investigate the aetiology of conditions such as hydrocephalus and dementia, where there is a high degree of clinical overlap and that share hypertension as a common risk factor for their onset.

**Conclusion**

In this work we provide the first application of the BCSFB-ASL technique to the rat brain, providing reference values for rates of BCSFB-mediated water delivery into the lateral
ventricles. The decreased rate of BCSFB-mediated arterial water delivery and altered CSF homeostasis in hypertensive subjects demonstrates the potential utility of the method to study the mechanisms that link systemic hypertension to downstream neurodegeneration. The observed functional impairment at the BCSFB suggests that the CP-BCSFB-CSF system may represent a key site of brain vulnerability to systemic hypertension.
6. Chapter 6 – Summary and Future Directions

a. Thesis Summary

The BCSFB, a unique interface between the systemic circulation and the brain, performs a broad range of roles to ensure CNS homeostasis. Historically, a lack of non-invasive, translatable methodologies has limited a thorough exploration of the BCSFB in *in-vivo* healthy and diseased brain states\(^9\),\(^{155}\). To try to address this need, the development of BCSFB-ASL has permitted the non-invasive measurement of rates of BCSBF-mediated water delivery into ventricular CSF, as a surrogate marker of BCSFB function\(^1\). The BCSFB-ASL methodology described and characterised by Evans *et al.* in the mouse brain provided a foundation for the work presented in this thesis\(^1\).

In Chapter 2, we introduced a method for the simultaneous recording of pharmacological perturbation of BCSFB function and cortical perfusion using interleaved echo-time ASL. Together these data, the first of such kind, highlight the value of this translational approach to capture simultaneous and differential pharmacological modulation of vessel tone at the BBB and BCSFB, and how this relationship may be modified in the ageing brain.

We then sought to reproduce and characterise the BCSFB-ASL MRI approach on the Bruker 9.4T system. Here, we successfully capitalised on the hardware and software improvements of the Bruker system to optimise several features of the acquisition: labelling efficiency, SNR, and temporal resolution for both single- and multi-TI approaches, thereby ensuring increased measurement precision in conjunction with minimised scan times.

Chapter 4 describes an investigation into the contributing sources and kinetics of signals arising from CSF regions densely vs sparsely populated with CP tissue. Alongside determining whether ASL signals from these regions can provide reliable water delivery values, these data aimed to also inform on other factors contributing to the appearance of long TE ASL signals in CSF regions distal to the caudal lateral ventricles. At this stage, we were unable to unambiguously determine whether non-zero water delivery rates reflected non-BCSFB-derived routes of labelled blood water delivery into distal CSF regions, or whether this was a result of rapid CSF mixing.
In order to non-invasively detect possible derangement of BCSFB function in the Spontaneous Hypertensive Rat (SHR), we utilised a multi-TI BCSFB-ASL approach. SHRs displayed a reduction in BCSFB function and alterations in CSF homeostasis, relative to normotensive controls. Our data highlights the potential for BCSFB-ASL to serve as a sensitive early biomarker for hypertension-driven neurodegeneration, in addition to investigating the mechanisms relating hypertension to neurodegenerative outcomes.

**b. Methodological improvements**

A key feature of BCSFB-ASL is the use of an ultra-long TE to isolate signal from ventricular CSF, whilst nulling signal contributions from surrounding tissue. Experiments in Chapter 3 were able to show an improved SNR at a lower echo time (TE = 180 ms), but partial volume effects (PVE) from surrounding brain tissues were more pronounced. Moving forward, the optimisation of SNR by lowering the TE parameter by a smaller increment (e.g. TE = 200 ms) would be beneficial in optimising the acquisition.

When acquiring multi-TI BCSFB-ASL data on the Bruker 9.4T system (e.g. Chapters 4 and 5), we noticed that early TI values provided ΔM values which were subject to elevated levels of random noise. In particular, when assessing differences in bolus arrival time to describe the kinetics of water delivery to brain-wide CSF spaces, noisy data at early TIs limited the extraction of precise arrival time values. Improvements in the imaging paradigm to reduce noise at these TI values would not only aid in a more thorough characterisation of kinetic signals at these CSF spaces, but also, by improving the model fitting accuracy, would be beneficial in studies where changes in arrival time provide a disease biomarker. For example, delays in arrival time have been shown to be characteristic of steno-occlusive cerebrovascular diseases \(^{230}\), and may be adversely affected by aging-related disorders, including dementia \(^{231}\).
c. **Physiological interpretation of BCSFB-ASL signal**

The BCSFB-ASL measurement primarily reflects changes in perfusion to the choroid plexus, convolved with the mass of the choroid plexus (a constant), and its permeability (extraction fraction). The phMRI experiments conducted with vasopressin in Chapter 2 highlight that we are primarily sensitive to changes in CP perfusion. Although providing an informative surrogate measure of BCSFB function, experiments aiming to elucidate the physiological underpinnings of the measurement will aid in a more comprehensive biological interpretation of the signal. To achieve this, the experimental approaches used will need to transition away from focusing on translatability and towards the utilisation of *ex-vivo* and more invasive methodologies, such as contrast-enhanced MRI, surgical procedures, and biochemical/histological assessment of choroid plexus tissue and the associated vasculature.

To provide further evidence towards BCSFB-ASL’s sensitivity to perfusion changes, it would be valuable to conduct contrast-enhanced MRI to better understand the contributions of permeability changes. Previous work using gadolinium-based contrast agents have shown the effect of ageing on BCSFB leakiness and homeostatic water exchange with DCE-MRI. Employing a similar DCE approach in conjunction with BCSBF-ASL would provide a correlation between the permeability and perfusion contributions, which would therefore improve the understanding of the underlying physiological changes detected by BCSFB-ASL.

In a similar manner, obtaining an invasive gold-standard standard measurement of perfusion to the choroid plexus using the microsphere approach can then further verify the degree of sensitivity provided by BCSFB-ASL to measure perfusion changes. These measures can then be correlated to BCSFB-ASL and DCE-MRI measures obtained previously in the same animals to provide a comprehensive outline of the sensitivity of the BCSFB-ASL approach. However, these measurements are challenging to perform and to my knowledge have yet to be conducted in the mouse brain, likely due to the difficult brain dissection process required.

Previous studies have employed invasive surgical approaches, i.e. the Masserman or Papenheimer techniques, to demonstrate that in human ageing and AD, there are reduced CSF secretion rates. Given that rates of CSF production have been proposed to be coupled to perfusion to the CP, by correlating BCSFB-ASL invasive measures to CSF secretion rates, we can begin to understand whether, and in what pathological scenarios, the
BCSFB-ASL signal is able to provide a surrogate non-invasive correlate of CSF secretion in a
readily translatable manner.

Two-photon microscopy imaging of the choroid plexus *in-vivo* and in tissue explants by the
Lehtinen group has provided a platform for investigating cellular events and subcellular
interactions at the BCSFB *in-vivo*. By combining this chronic two-photon imaging using
various molecular markers, with BCSBF-ASL, we would be able to begin pinpointing the
cellular events which drive changes in BCSFB-ASL signals.

**d. Applications to study BCSFB function in health and disease**

The phMRI experiments conducted in this work provide a platform for the investigation of
pharmacological modulation of BCSFB function. Given the optimisation of the BCSFB-ASL
methodology on the Bruker 9.4T system, the effects of selected challenges can be
investigated to a higher degree of precision and at an increased temporal resolution. This
would be useful to gain an increased confidence when investigating the effects of hypercapnia
on BCSFB-ASL measurements, where the current dataset presented in Chapter 2 suffers from
greater levels of noise due to the relatively small signals being detected. Furthermore, we can
begin to investigate chronic pharmacological modulation of BCSFB function. For example, in
the clinical setting, hypertension is treated using a range of drugs, e.g. angiotensin converting
enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARB). By dosing SHRs with
ACE or ARB drugs over the course of several weeks, we can determine whether BCSFB
function can be recovered in a sustained manner.

The work within this thesis has provided further evidence that BCSFB function is impaired in
altered brain states associated with neurodegeneration. For example, aged mice display a
decreased BCSFB reactivity to a vasopressin challenge (Chapter 2), and neurodegeneration-
prone SHRs were shown to have markedly impaired baseline BCSFB function (Chapter 5). To
supplement this work, a longitudinal study could inform on the timeline of BCSFB functional
decline, thereby adding critical mechanistic information on downstream neurodegeneration.

For example, a study across the lifespan of SHRs could determine the age at which BCSFB
functional decline first develops. Alternatively, a multi-timepoint study in SHRs of different
ages could be used. By combining measures of i) cerebrovascular health biomarkers (BCSFB
function, cortical CBF) with ii) behavioural assessments of cognitive impairment, and iii) ex-
vivo measurements of neurodegenerative staging, we can gauge the effectiveness of BCSFB-ASL in determining neurodegenerative outcomes relative to other biomarkers of cerebrovascular health (e.g. cortical CBF). Additionally, by using a tail-cuff to measure blood pressure (BP) non-invasively prior to commencing the BCSFB-ASL measurement, we would be able to correlate the extent of hypertensive damage to the choroid plexus with absolute BP values.

**e. Conclusion**

This thesis has demonstrated the value of a BCSFB-ASL approach in detecting functional derangement of the BCSFB in altered brain states, namely ageing and hypertension, which reiterates that the BCSFB provides a rich source of biomarkers, as well as mechanistic understanding of how functional impairment may seed pathological processes in a variety of pathologies. Beyond the application to other disease states, the improved acquisition on the Bruker system has broadened the scope of applications. For example, we are now able to investigate brain-wide ultra-long TE ASL signals which, in turn, may permit the characterising of signal kinetics to elucidate novel mechanisms of water delivery in the brain.
7. Bibliography


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