Tampereen yliopisto

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AN INTRODUCTION TO CHOROID PLEXUSES AND THEIR ROLE IN THE GLYMPHATIC SYSTEM

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ABSTRACT

Emma Väätäinen: An introduction to choroid plexuses and their role in the glymphatic system
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Choroid plexuses are brain structures that contribute to the waste-clearance of the brain by secreting cerebrospinal fluid. In humans, four choroid plexuses are located in each of the four brain ventricles. The choroid plexuses work as a part of the glymphatic system, whose purpose is to clear excessive neuropeptides and other metabolic waste products from the central nervous system with cerebrospinal fluid circulation. The glymphatic system works constantly, with a significant acceleration during sleep.

A choroid plexus consists of microvillous epithelial cells, which fold around a highly vascularised connective tissue, creating a lobulated, leafy-like structure. The epithelium faces the ventricle, which is filled with cerebrospinal fluid. The choroid plexus epithelial cells and the tight junctions connecting them form the blood-cerebrospinal fluid barrier, which restricts nonspecific passage of solutes from capillaries into the cerebrospinal fluid. The choroid plexus epithelial cells contain multiple solute transporters, which allow a selective passage of solutes through the epithelium. Immune cells also traffic across the blood-cerebrospinal fluid barrier to access the central nervous system, making the choroid plexuses central participants in neuroinflammatory responses.

Multiple neurological, including neuroinflammatory, -degenerative- and -psychiatric diseases cause several morphological and functional changes in the choroid plexuses. A common pathology reported in multiple diseases is the volumetric enlargement of the plexuses, which is often accompanied by chronic low-grade neuroinflammation. The structural changes that occur in the plexuses during healthy ageing are not fully understood, and they often overlap with the modifications seen in diseased ageing. This makes it difficult to determine the line between pathological and non-pathological structural modifications.

The aim of this project was to introduce the choroid plexuses as key contributors in the upkeep of central nervous system homeostasis, and to discuss their known role in neurological diseases to date. An additional assessment of histological samples from the Tampere Sudden Death Study was performed to determine the number of cases that included a choroid plexus sample. Further studies to detect and measure age-related changes can be executed using these samples.

Keywords: Choroid plexus, Glymphatic system, Cerebrospinal fluid, Blood-cerebrospinal fluid barrier

TIIVISTELMÄ

Emma Väätäinen: Johdanto aivokammioiden suonipunoksiin ja niiden tehtävään osana glymfaattista järjestelmää
Kandidaatintutkielma
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Aivokammioiden suonipunokset ovat aivokammioissa sijaitsevia rakenteita, jotka osallistuvat keskushermoston puhdistukseen erittämällä aivo-selkäydinnestettä. Ihmisen aivoissa sijaitsee neljä aivokammiota, joiden seinämistä suonipunokset ulkonevat. Aivokammioiden suonipunokset toimivat osana glymfaattista järjestelmää, jonka tavoite on puhdistaa keskushermostosta sinne kerääntyvät aineenvaihdunnan jätetuotteet aivo-selkäydinnesteen virtauksen avulla. Glymfaattisen järjestelmän toiminta on jatkuvaa ja tehostuu huomattavasti unen aikana.

Aivokammioiden suonipunoksissa epiteelisolut ympäröivät runsaasti verisuonia sisältävää sidekudosta muodostaen lehdenomaisen rakenteen. Suonipunosten epiteelisolut suuntautuvat kohti aivokammioita, jotka ovat täyttyneet aivo-selkäydinnesteellä. Aivokammioiden suonipunosten epiteelisolut ja niitä yhdistävät tiiviit liitokset muodostavat esteen, joka rajoittaa molekyylien vapaata liikettä verenkierron ja aivo-selkäydinnesteen välillä. Epiteelisolujen kalvoilla sijaitsee useita kuljetusproteiineja, jotka mahdollistavat veren liukoisten molekyylien valikoivan kuljetuksen suonipunosten epiteelien läpi. Myös useat immuunijärjestelmän solut kulkevat suonipunosten epiteelin läpi keskushermostoon, minkä vuoksi suonipunoksilla on keskeinen rooli keskushermoston tulehdusreaktioissa.

Useat neurologiset sairaudet, kuten hermorappeumataudit, psykiatriset sairaudet ja hermoston tulehdustilat, aiheuttavat rakenteellisia muutoksia aivokammioiden suonipunoksissa. Muun muassa suonipunosten tilavuuden on raportoitu kasvavan useiden keskushermoston sairauksien yhteydessä. Tilavuuden kasvuun liittyy usein pitkittynyt matala-asteinen keskushermoston tulehdustila. Terveestä ikääntymisestä aiheutuvat muutokset aivokammioiden suonipunosten rakenteissa ovat puutteellisesti ymmärrettyjä ja osittain yhteneviä sairauksien seurauksena kehittyvien muutosten kanssa. Tämän vuoksi patologisten ja ei-patologisten muutosten välinen ero on usein epäselvä.

Tämän työn tavoitteena oli etsiä tietoa aivokammioiden suonipunosten merkityksestä keskushermoston dynaamisen tasapainotilan ylläpitämisessä ja koota yhteen viimeisin tutkimustieto niiden osallisuudesta yleisissä neurologisissa sairauksissa. Täydentävä mikroskopointityö Tampere Sudden Death Study -tutkimuksen histologisilla näytteillä suoritettiin aivokammion suonipunosta sisältävien näytteiden lukumäärän selvittämiseksi. Näytteitä voidaan käyttää jatkotutkimuksissa, joissa pyritään havaitsemaan ja mittaamaan tavallisesta ikääntymisestä johtuvia aivokammioiden suonipunosten rakenteellisia muutoksia.

Avainsanat: Aivokammion suonipunos, Glymfaattinen järjestelmä, Aivo-selkäydinneste, Veri-aivo-selkäydinneste-este

PREFACE

This thesis was conducted as a part of my bachelor studies in the Biotechnology and Biomedical Engineering programme. I would like to thank my thesis supervisor Eloise Mikkonen for her great guidance, patience and support throughout the writing process. I would also like to thank my mother, who attempted to read the unfinished thesis without having much knowledge in the biomedical field.

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Emma Väätäinen

CONTENTS

1. INTRODUCTION

The glymphatic system was first introduced in 2012 as a macroscopic waste clearance system that utilises the flow of the cerebrospinal fluid to pick up metabolic waste products from the brain parenchyma and provide nutrients, growth factors and neuromodulators to neurones. Since its introduction, the glymphatic system has been the centre of attention in research on neurodegenerative diseases, as the system has been seen to deteriorate with ageing, leading to a compromised homeostasis of the central nervous system and the accumulation of neuropeptides. (Jessen *et al.*, 2015)

Highly vascularised, leafy-like structures called choroid plexuses protrude from the ventricular walls of each brain ventricle. The choroid plexuses serve multiple important functions, including the secretion of cerebrospinal fluid, regulation of leukocyte traffic and formation of the blood-cerebrospinal fluid barrier. Their discovery as the source of cerebrospinal fluid dates to 1664, but their involvement in the progression of neurological diseases is still only partly known to this day. Healthy and diseased ageing, as well as the development of several neuropsychiatric diseases, are associated with structural and functional modifications of the choroid plexus tissue. The causalities of these modifications are however often unknown and difficult to distinguish from nonpathological modifications of ageing. The research on neurological diseases and their treatment will benefit from further studies on the choroid plexuses and the blood-cerebrospinal fluid barrier they form. (Saunders *et al.*, 2023)

2. THE GLYMPHATIC SYSTEM

As a crucial part of tissue function, unnecessary, often toxic metabolic waste products need to be discarded to maintain tissue homeostasis. In the central nervous system (CNS), this system of waste-clearance is called the glymphatic system. The high metabolic activity of neurones requires an efficient way of waste clearance to avoid an accumulation of metabolic waste products. The waste products include clinically significant proteins such as amyloid beta, tau and alpha-synuclein. The main components operating within the glymphatic system include the choroid plexuses, perivascular spaces, astrocytes and the aquaporin channels at their endfeet, the cerebrospinal fluid (CSF) and the interstitial fluid (ISF) it mixes with, and the efflux routes for discarding waste.

In addition to waste clearance, the glymphatic system works as a way to deliver nutrients, neuromodulators, growth factors and immune cells to the CNS. The system works continuously, but accelerates significantly during sleep. (Jessen *et al.*, 2015)

In peripheral organs, metabolic cellular waste, along with excess tissue fluids, are brought back to the blood circulation via the lymphatic vessel network. The blood-brain barrier (BBB), however, isolates the CNS from the peripheral lymphatic network, which is why it requires its own waste-clearance system (Jessen *et al.*, 2015). The BBB is formed from the tight junctions between blood vessel endothelial cells, and its purpose is to keep the brain parenchyma separate from the blood circulation. Another barrier called the blood-cerebrospinal fluid barrier (BCSFB) is constructed from tight junctions linking the choroid plexus epithelial cells (Jessen *et al.*, 2015). The BCSFB keeps blood circulation separate from the CSF. The BBB is a much tighter barrier compared to the BCSFB, which is often described as leaky (Jessen *et al.*, 2015; Praetorius and Damkier, 2017).

There are four ventricles in the brain; two lateral ventricles, the third ventricle and the fourth ventricle, which each possess a choroid plexus (CP). The plexuses in the lateral ventricles secrete CSF, which then flows through the intraventricular foramen to the third ventricle, and further through the cerebral aqueduct to the fourth ventricle. The third and fourth ventricles both also contain plexuses that secrete more CSF. CSF flow then continues into the subarachnoid space positioned between two meninges. In the brain parenchyma, astrocytes position their extensions, or legs, loosely around arteries, creating periarterial spaces between the sheets of astrocyte endfeet and arteries. The CSF flow continues from the subarachnoid space into these periarterial spaces and further flows through or between the astrocyte endfeet via aquaporin 4 (AQP4) channels into the interstitial space, thus “bathing” the brain parenchyma. In the interstitial space, CSF flow combines with the ISF, carrying nutrients to neurones and picking up metabolic waste products. The CSF-ISF efflux occurs via perivenous spaces into various drainage routes. The CSF draining into the peripheral lymphatic system occurs via the olfactory bulb and along the spinal and cranial nerves. (Jessen *et al.*, 2015)

3. CHOROID PLEXUSES

3.1 Structure

Choroid plexuses (CPs) are located in the four brain ventricles. The location of the four plexuses and the ventricular spaces is illustrated in Figure 1. They are leaf-like structures that arise

from the ventricle-lining ependyma and float in the cerebrospinal fluid. The macroscopic structures of the CPs of each ventricle vary notably. The lateral ventricle CPs are thin and wavey in structure, and they have anterior and posterior domains. The fourth ventricle CP is more complex in structure, lobulated and the most compact. The third ventricle CP protrudes from the roof of the ventricle, is the smallest and has an intermediate structure between the other plexuses. (Strazielle and Ghersi-Egea, 2000; Wolburg and Paulus, 2010; Saunders *et al.*, 2023)

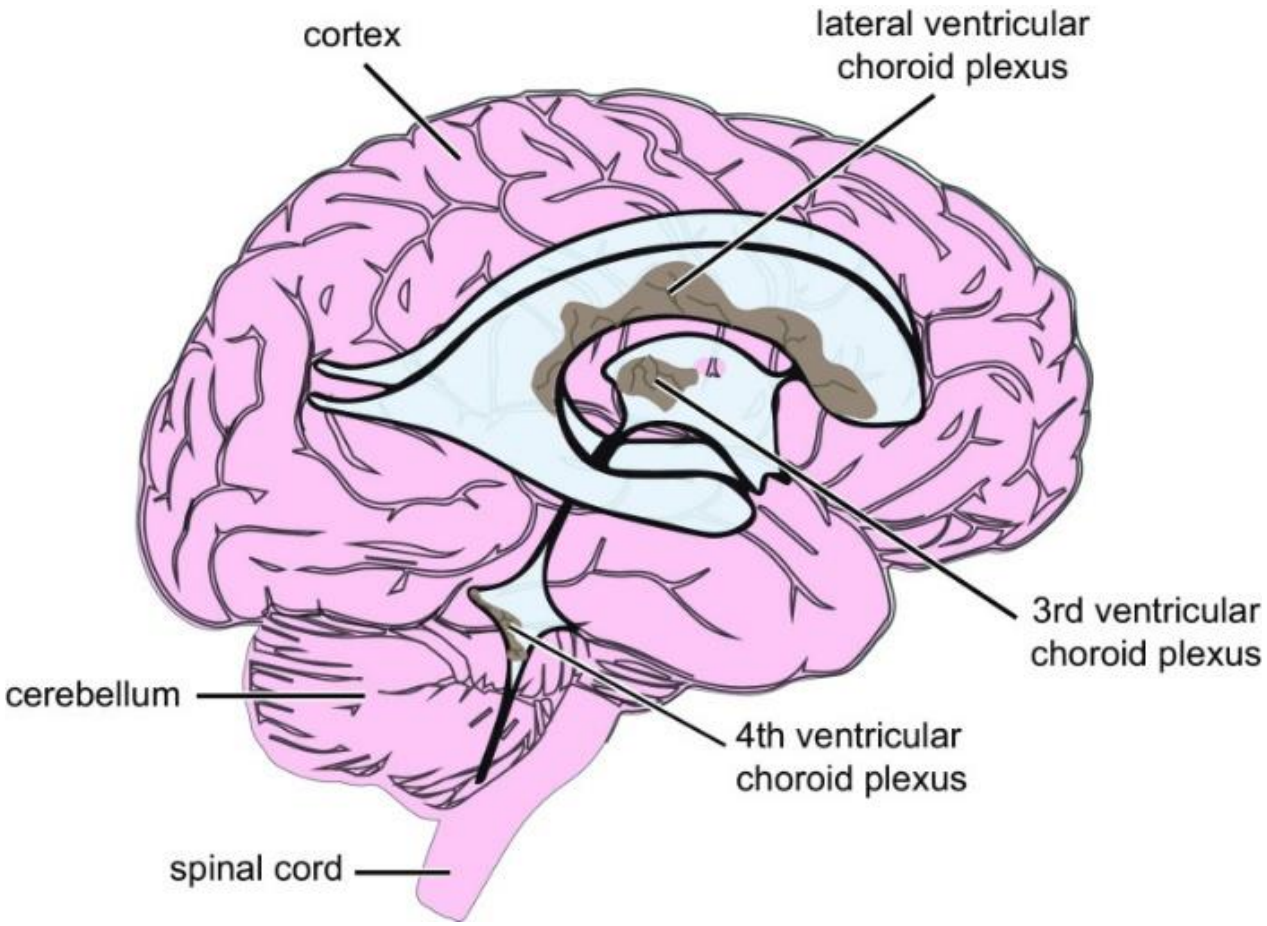

Figure 1: *The location of the four choroid plexuses (Saunders* et al.*, 2023).*

On a microscopic level, however, the CPs of the different ventricles consist of similar components. The ependyma lining the ventricle walls continues as an ependymal epithelium of the CP. This ependymal epithelium further continues into the choroid plexus epithelium, which folds into villous fronds around highly vascularised connective tissue (Strazielle and Ghersi-Egea, 2000). Choroid plexus epithelial cells (CPE cells) are the dominant cell type in the CP structure (Hofman and Chen, 2015). In addition to epithelial cells, other cell types generally found in the CPs during development are endothelial, mesenchymal (mural, fibroblast, pericyte and vascular smooth muscle cells), immune, neuronal and glialike cells (Saunders *et al.*, 2023). The cellular structure of the CPs can be seen in Figure 2.

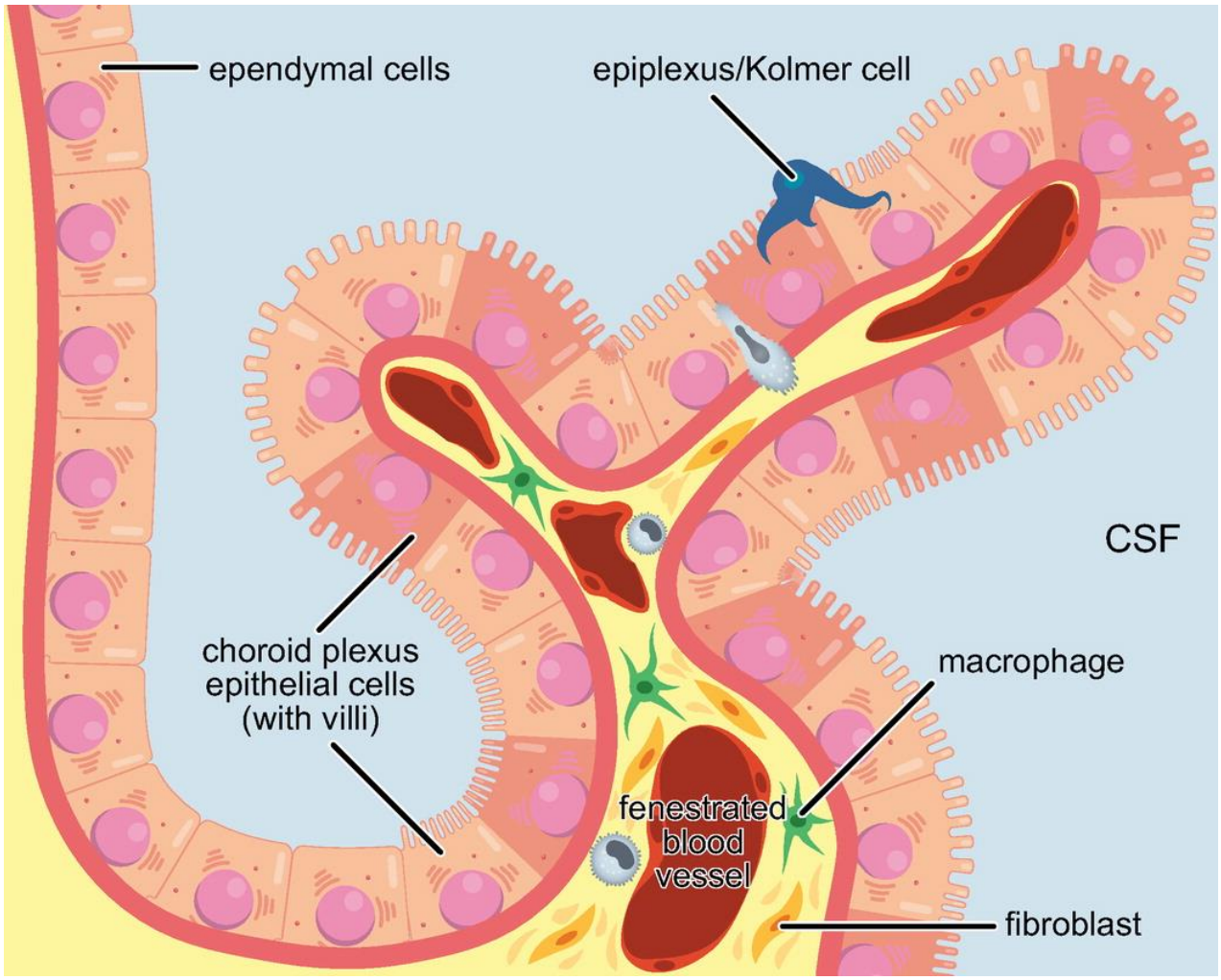

Figure 2: *The cellular structure of a choroid plexus (Saunders* et al*., 2023).*

3.1.1 Choroid plexus epithelial cells

According to estimations based on cell counts, the CPE cells make up 61-69% of all CP cells, and they are responsible for the majority of CSF formation (Hofman and Chen, 2015; Saunders *et al.*, 2023). CPE cells contain a relatively large number of mitochondria, which is a common feature of secreting or reabsorbing epithelial cells (Strazielle and Ghersi-Egea, 2000; Damkier *et al.*, 2013). The CPE cells also carry abundant Golgi apparatuses and have plenty of cytosolic vesicles, which appear larger on the luminal side of the cells (Damkier *et al*., 2013).

The apical CSF-facing sides of CPE cells are densely and irregularly covered with microvilli to enhance the surface area of the cell membranes (Strazielle and Ghersi-Egea, 2000; Damkier *et al.*, 2013). Along the microvilli, many CPE cells contain 8-10 cilia, the physiological function of which is not yet completely understood. The cilia on CPE cells have presented both motile and immotile configurations (Saunders *et al.*, 2023). The motile cilia do not seem to direct the flow of CSF, but it

has been suggested that they stir the secreted CSF to prevent layering and function as mechanosensors during foetal ventricular development (Saunders *et al.*, 2023). The absence or defectiveness of these cilia has been shown to dramatically alter CP function. Mice with defective cilia structures display changes in CSF composition and pH, resulting in the development of hydrocephalus (Damkier *et al.*, 2013).

The types of CPE cells vary slightly with the presence of dark epithelial cells. Dark CPE cells were discovered in histological preparations in the late nineteenth century, but their purpose remains puzzling to this day. The dark cells differentiate from the rest of the CPE cells with a greater density of cytoplasm, nuclei and microvilli, as well as a higher number of mitochondria. In some *in vivo* and *in vitro* studies, the number of dark cells in the epithelium has been altered when administered with fibroblast growth factor 2 or arginine vasopressin, with fibroblast growth factor 2 also causing a reduction in CSF secretion. However, the mechanisms and pathways underlying these phenomena are unclear. (Saunders *et al.*, 2023)

The CPE cells differ from the neighbouring ependymal cells with the presence of apical tight junctions, which are essential for the formation of the BSCFB. The role of the tight junctions connecting the epithelial cells is to regulate the paracellular passage of water-soluble molecules and maintain electrical resistance across the epithelium (Solár *et al.*, 2020). The tight junctions express several types of claudin and occludin, which are connected to intracellular actin filaments via three types of zonulin proteins (Solár *et al.*, 2020). The expression patterns of claudins in CPE cells mimic the tight junction protein compositions present in proximal tubules that allow water to pass through epithelium (Damkier *et al.*, 2013).

3.1.2 Connective tissue

The CPE cells are connected to a basal lamina, which separates them from the connective tissue underneath. The CP connective tissue consists of mesenchymal-like fibroblasts, often called CP stromal cells, as well as capillaries that are relatively large compared to others found in the brain (Hofman and Chen, 2015). The endothelial cells of CP capillaries are porous, often referred to as fenestrated, which permits the passage of most small hydrophilic molecules into the interstitial fluid of the connective tissue (Saunders *et al.*, 2023). The extracellular space of the CP stroma and blood circulation are not separated by a barrier, since the endothelial cells lack tight junctions (Hofman and Chen, 2015). A group of macrophages and other immune cells are also resident within the CPs. Their role is discussed later in the “Choroid plexus in disease” section.

3.2 Development of the choroid plexuses

During embryonic development, each of the four CPs develop independently. Following the closure and expansion of the neural tube, the plexuses start to emerge from its roof plate (Carpenter, 2015; Saunders *et al.*, 2023). The timing of CP development varies across species and doesn't seem to correlate with the stage of gestation or the time of birth (Saunders *et al.*, 2023). In humans, the first CP to appear is the fourth ventricular plexus at around 6 weeks of gestation. The lateral ventricular plexuses follow at 7 weeks, and the third ventricular plexus at 8 weeks of gestation (Saunders *et al.*, 2023). The timing of CP development varies in literature, due to the differences in how gestational length is defined and the difficulty in determining when fertilisation occurs (Saunders *et al.*, 2023). The CPE cells are derived from neuroepithelium, and the CP stromal cells have mesenchymal origins (Carpenter, 2015).

The development of the lateral ventricular plexuses in humans can be divided into four stages, where the developing CPE cells can be separated into six types. The four stages have been determined by histological criteria and the varying presence of glycogen in the epithelial cells. The six epithelial cell types are divided according to shape and nuclear position. (Saunders *et al.*, 2023)

In stage I, at 7 weeks of gestation, the neuroepithelium continues as a pseudostratified epithelium. This refers to a stage where the epithelial cells are closely packed and of different sizes, making the epithelium look like it has multiple layers, even though there is only one. The CPE cells have centrally positioned nuclei and they still lack glycogen. Vascularisation also starts to appear at this stage. (Saunders *et al.*, 2023)

Stage II starts around the 9th week of gestation and is characterised by volumetric growth of the CPs from their bases. At around the 11th week, the CPs occupy most of the lateral ventricular spaces, after which the ventricles themselves start to expand (Saunders *et al.*, 2023). The pseudostratified epithelium mostly becomes low columnar epithelium, and it starts to lobulate. The CPE cells fill with glycogen and their nuclei move to the ventricular sides of the cells (Saunders *et al.*, 2023). New cells proliferate at the base of the plexuses, where the epithelia lack glycogen and are structured in a pseudostratified manner (Saunders *et al.*, 2023). As the morphological differentiation of the epithelial cells continues, the expression of transthyretin appears. It acts as a biological marker of a committed CPE cell, as the CPE cells are responsible for its production (Carpenter, 2015).

In stage III the CPE cells are cuboidal, their nuclei are positioned centrally or apically and the glycogen content declines. In stage IV, the epithelium is cuboidal or squamous, the nuclei have central or basal positions and the glycogen is gone. (Saunders *et al.*, 2023)

The research on CP development has focussed largely on the plexuses of the lateral ventricles, and therefore information on the third and fourth ventricular CP development is very limited. The third ventricle plexus is seen to undergo the same four stages described earlier during its development, but less than six types of epithelial cells are present. During the development of the fourth ventricular plexus, the six cell types are present, but there are no distinct stages in the development. (Saunders *et al.*, 2023)

3.3 Choroid plexuses secrete cerebrospinal fluid

The CSF is a colourless liquid that consists mainly of ions, nutrients and water. The CSF surrounds the CNS and participates in several important processes, such as CNS protection and waste clearance. The central role of the CSF in CNS homeostasis orders for its continuous formation, with a rate of 10-20 mL/h, adding up to 600 mL secreted in a day. The ventricular system holds 150 mL of CSF, with around 25 mL in the ventricles and 125 mL in the subarachnoid spaces, meaning that the CSF is renewed 3-4 times daily (Strazielle and Ghersi-Egea, 2000; Damkier *et al.,* 2013; Jessen *et al.*, 2015). The majority, around 50-90%, of CSF formation is performed by the CPs, and the rest comes from the ISF generated by the BBB (Damkier *et al.*, 2013; Jessen *et al.*, 2015; Solár *et al.*, 2020). In addition to its role in the glymphatic system's waste clearance, the CSF serves a few other functions, such as cushioning the brain to physically protect it from traumatic injuries (Damkier *et al*., 2013)

Ions, nutrients and water are the main components of the CSF, but the fluid also contains neuromodulators, growth factors and immune cells (Jessen *et al.*, 2015). The formation of the CSF depends mostly on the selective transcellular transport of Na^+, Cl^-, K^+, Ca^{2+}, HCO_3^- and water through the choroid plexus epithelial cells (Damkier *et al.*, 2013; Jessen *et al.*, 2015). The components arrive from the fenestrated CP capillaries into the interstitial space of CP connective tissue, where transporters on the basolateral side of the epithelial cells then pick them up for transportation across the epithelium. The ions move through several types of transporters, which in contrast to other secretory epithelial cells, are more concentrated on the luminal surface of the CPE cells instead of the basolateral (Damkier *et al.*, 2013; Solár *et al.*, 2020).

The ion concentrations in the CSF resemble the concentrations in blood plasma with minor, but significant differences. The CSF has a larger concentration of Cl^- and smaller concentrations

of K^+, Ca^{2+} and HCO_3^- compared to plasma (Damkier *et al.*, 2013). Even though they share similarities in ion composition, the CPs actively form and regulate the CSF composition and volume, and therefore the CSF is not just an ultrafiltrate of plasma. This has been evidenced by experiments, where the concentrations of K^+, HCO_3^- and Ca^{2+} in plasma have been experimentally altered, but the concentrations of the ions have remained constant in the CSF (Damkier *et al.*, 2013).

3.3.1 Water transport

The CSF has a high water content of approximately 99% (Damkier *et al.*, 2013). Transcellular water fluxes across the choroid plexus epithelium occur through aquaporin 1 channels (AQP1), which are located in the apical and basolateral membranes of the CPE cells, with a higher abundance on the apical side (Jessen *et al.*, 2015; Praetorius and Damkier, 2017). Water transport seems to be connected to transcellular Na^+ transport and is therefore believed to be driven by osmotic force (Praetorius and Damkier, 2017).

Other aquaporins besides AQP1 have also been reported to be present in the CPE cells, but the role of the AQP1 channel is the most significant and well-known. The inhibition of AQP1 channels in mice has been documented to cause a significant reduction in luminal water permeability of 80%, and a reduction in CSF secretion by 35% (Damkier *et al.*, 2013; Jessen *et al.*, 2015). In addition to AQP1, AQP4 channels have recently been suggested to have a role in CSF production that fluctuates with age. In a recent study by Deffner et al., the presence of AQP4 channels was documented in the basolateral membrane of CPE cells of human donors with old age (74-91 years) (Deffner *et al.*, 2022). This may be relevant, as the presence of the AQP4 channels in human CPE cells is not as common as the expression of AQP1 channels. In their paper, Deffner et al. proposed that the presence of AQP4 channels may compensate for the loss of AQP1 channels in ageing.

3.3.2 Cation transport: Na^+, K^+ and Ca^{2+}

Na^+ is deemed as the most important ion transported through the CP epithelium. Its transport is quantitatively highest and provides a driving force for CSF secretion (Damkier *et al.*, 2013). The majority of Na^+ transport happens through a Na^+-K^+-ATPase, which is therefore one of the most important transporters in CSF secretion (Damkier *et al.*, 2013; Jessen *et al.*, 2015; Solár *et al.*, 2020). Contrary to most secretory epithelia where the Na^+-K^+-ATPases are found at the basolateral membranes of secretory cells, the Na^+-K^+-ATPases in the CPE cells are located at the luminal membrane (Damkier *et al.*, 2013). They pump Na^+ into the CSF whilst simultaneously bringing K^+ into the cell. The resulting concentration gradients of Na^+ and K^+ drive the other ion transporters (Damkier *et al.*, 2013). The role of Na^+-K^+-ATPases is central to the overall CSF formation. This

has been shown by experiments, where inhibition of the transporters with ouabain has reduced the total CSF secretion by 50-60% (Jessen *et al.*, 2015; Praetorius and Damkier, 2017). Na^+ transport across the CP epithelium occurs through other channels as well, but their contribution to the overall Na^+ traffic is not as significant as that of Na^+-K^+-ATPases (Damkier *et al.*, 2013).

The K^+ concentration in the CSF is precisely regulated (Damkier *et al.*, 2013). The K^+ ions absorbed into the CPE cells by Na^+-K^+-ATPases are mostly recycled back by K^+ channels to avoid the accumulation of K^+ and maintain a negative intracellular membrane potential (Damkier *et al.*, 2013; Praetorius and Damkier, 2017). A K^+/Cl^- cotransporter called KCC4 aids in the recycling of K^+ at the luminal membrane, and a KCC3 cotransporter transports K^+ through the basolateral membrane (Praetorius and Damkier, 2017).

Ca^{2+} ions play a part in multiple important processes in the CNS, like controlling neurotransmitter release, modulating the activity of ion channels and contributing to depolarising currents (Damkier *et al.*, 2013). In addition, Ca^{2+} ions regulate long-term potentiation, cell death and neuronal plasticity. Intracellular Ca^{2+} levels in the CNS are strictly regulated and the stability of this relies on the long-term control of CSF Ca^{2+} levels. Ca^{2+} transport into CSF is regulated by both the BBB and the BCSFB. Transcellular Ca^{2+} transport across CP epithelium is likely to function via a Ca^{2+}-ATPase, a Ca^{2+}-permeant cation conductance and exocytosis (Praetorius and Damkier, 2017).

3.3.3 Anion transport: HCO_3^- and Cl^-

The transport of HCO_3^- ions is also essential for the overall formation of CSF (Damkier *et al.*, 2013). The HCO_3^- formation from water and carbon dioxide is catalysed by intracellular enzymes called carbonic anhydrases, and when they have been experimentally inhibited with acetazolamide, the overall CSF formation has reduced by 50-100% (Praetorius and Damkier, 2017). Additional HCO_3^- is imported from blood and is exported into the CSF via several transporters, including multiple Na^+-HCO_3^- cotransporters (Damkier *et al.*, 2013).

Cl^- transport across CPE cells occurs through anion exchangers in the basolateral membrane, and through Cl^- channels and cotransporters in the luminal membrane (Damkier *et al.*, 2013; Praetorius and Damkier, 2017). The uptake of Cl^- from blood occurs via Cl^-/HCO_3^- exchangers, which connect the transport of the two ions (Praetorius and Damkier, 2017). The pathways for Cl^- transport, sometimes connected to $HCO3^-$ transport, are inhibited by the stilbene derivate DIDS, which causes an overall reduction in CSF secretion (Damkier *et al.*, 2013).

4. CHOROID PLEXUSES IN DISEASE

4.1 Choroid plexuses in neuroinflammation

The CPs link the immune systems of the CNS and the peripheral body by allowing the entry of immune cells into the CNS across the BCSFB (Saunders *et al.*, 2023). Pathogens can also access the CNS by crossing the BCSFB, which can lead to infectious diseases in the CNS, such as meningitis and meningoencephalitis (Schwerk *et al.*, 2015). During a CNS infection, the CPs can respond by producing chemokines and cytokines, and altering the BCSFB barrier function, this way recruiting and trafficking immune cells, such as macrophages, neutrophils and T lymphocytes, across the BCSFB into the CNS (Schwerk *et al.*, 2015).

4.1.1 Barrier crossing by pathogens

Pathogens (bacteria, viruses, fungi and parasites) enter the CNS either across the BBB or the BCSFB. They can cross the BCSFB employing a few different strategies. Some pathogens penetrate the CP cells to move transcellularly through them. Others open the tight junctions between the CPE cells that form the BCSFB to cross the barrier paracellularly. Pathogens can also hijack a phagocytic host cell, a neutrophil for example, and use it in a “Trojan horse” manner to enter the CNS. (Schwerk *et al.*, 2015)

A prime example of a pathogen that rides across the BCSFB within macrophages in a “Trojan horse” manner is the human immunodeficiency virus (HIV). HIV infects the CPs by residing in monocytes and dendritic-like cells in the CP stroma (Schwerk *et al.*, 2015; Saunders *et al.*, 2023). HIV has deleterious effects on the immune system and thus people with an HIV infection often have other concurrent infections as well, which makes it difficult to determine how exactly the CPs respond to the infection (Saunders *et al.*, 2023).

As another example, *Streptococcus suis*, a gram-positive bacterium, is known to cause several changes in CP tissue. *S. suis* disrupts the BCSFB with a rearrangement of the tight junction proteins occludin, ZO-1 and claudin-1. During an *S. suis* infection the CPE cells experience an intracellular restructuring of actin, which forms bundles in the basolateral cell compartment causing cells to flatten. The infection causes an upregulation of genes in inflammatory cascades, such as the genes encoding several inflammatory molecules. *S. suis* also induces cell death in the CPE cells by apoptosis and necrosis, resulting in further alterations in the BCSFB. *S. suis* transport into the CNS

happens mainly transcellularly across CPE cells inside endocytic and exocytic vacuoles, but it might also utilise the “Trojan horse” and paracellular transport methods. (Solár *et al.*, 2020)

4.1.2 Immune cells in the choroid plexuses

The CPs are accompanied by resident immune cells. The immune cell types found in the developing CPs include macrophages, B cells, lymphocytes, basophils, mast cells, dendritic cells, neutrophils and monocytes (Saunders *et al.*, 2023). The majority of the resident immune cells are macrophages, which stay mostly in the CP connective tissues (Saunders *et al.*, 2023). The macrophages respond rapidly to inflammatory signals and elongate along the perivascular spaces of CP capillaries. Specialised macrophages called Kolmer cells are found on the microvillous CSF-facing surface of the CPE cells. They present phagocytic activity and play a role in CNS immunological responses (Damkier *et al.*, 2013; Saunders *et al.*, 2023).

During an inflammatory response, the CPs secrete chemokines and cytokines, such as interleukin 6, interleukin 8 and tumour necrosis factor α, to recruit immune cells into the CNS across the BCSFB (Schwerk *et al.*, 2015). The CPs also upregulate cell adhesion molecules like intercellular adhesion molecule (ICAM) 1 and vascular cell adhesion molecule (VCAM) 1, which interact with immune cell integrins on the cell surface to allow their trafficking on the epithelia (Schwerk *et al.*, 2015). The CPs interact with cytokines released by immune cells, which further intensifies the CNS inflammatory response (Saunders *et al.*, 2023). Immune cells have been documented to prefer crossing the BCSFB transcellularly, but evidence for paracellular immune cell traffic has also been found (Schwerk *et al.*, 2015). The CPs also have the ability to adjust their inflammatory responses by decreasing cytokine release during chronic inflammation (Saunders *et al.*, 2023).

The involvement of the CPs in immune cell trafficking during infectious diseases can cause complications in the CP tissue. Choroid plexitis, an inflammation of the CPs, is a rare complication of cryptococcal invasion. Enteroviral meningitis on the other hand has been observed to cause apoptosis of CP cells in mice. Additionally, the entrance of the immune cells into the CNS via the CPs is also partly responsible for cellular damage and degradation during infectious diseases in the brain. (Schwerk *et al.*, 2015)

4.2 Choroid plexuses in neurodegenerative diseases

The glymphatic system has received a lot of research attention since its discovery in terms of neurodegenerative diseases. All neurodegenerative diseases, including the most common and well-known Alzheimer’s (AD) and Parkinson’s (PD) diseases, share the characteristic of neurone loss during disease progression. Neurone loss leads to a variety of symptoms depending on the

disease, such as cognition impairment, loss of motor function and sleep problems (Gião *et al.*, 2022). The changes that occur in the CP tissues during disease progression are most documented in AD, as the disease has received substantial research attention since the discovery of the glymphatic system.

4.2.1 Alzheimer's disease

The CPs undergo several morphological changes in AD. A flattening of the CPE cells and a thickening of their basement membrane happen naturally as the CPs age. The CPE cells of AD patients, however, appear significantly flatter and their basement membrane thicker compared to age-matched controls (Gião *et al.*, 2022). Amyloid beta (Aβ) is a purportedly neurotoxic protein that is normally cleared from the CNS by the glymphatic system. Its overproduction and accumulation in the CNS due to reduced glymphatic function has been declared as the principal contributor to developing AD (Gião *et al.*, 2022). Aβ accumulates not only in the extracellular space of neurones, but also inside CPE cells, which disrupts the proper functioning of the plexuses by causing oxidative stress, mitochondrial dysfunction and structural alterations in the epithelial cells (Gião *et al.*, 2022; Municio *et al.*, 2023; Saunders *et al.*, 2023). The intracellular Aβ shrinks the nuclei and overall volume of CPE cells, which results in reduced CSF secretion by the plexus, further impairing the glymphatic system's ability to clear excess Aβ from the CNS (Gião *et al.*, 2022). The Aβ accumulation in the CPE cells also causes a downregulation in tight junction protein expression and an increase in matrix metalloprotease expression leading to a degradation of extracellular matrix proteins (Gião *et al.*, 2022). This leads to impairments in the BCSFB, as paracellular transport increases and becomes less specific, further resulting in compromised CSF homeostasis (Gião *et al.*, 2022).

An increased abundance of intracellular inclusions called Biondi ring tangles is documented in the CPE cells of AD patients (Gião *et al.*, 2022). Biondi rings in CPE cells are aggregations of tau protein, fibronectin, P component and ubiquitin, and their abundance causes structural damage to the cell membranes of CPE cells (Gião *et al.*, 2022). Biondi ring tangles appear in healthy-aged CPs as well, but their presence is increased in AD plexuses (Gião *et al.*, 2022; Saunders *et al.*, 2023).

Oxidative stress is one of the earliest events seen in the progression of AD (Gião *et al.*, 2022). Gene expression analyses have shown the CP cells in AD experience oxidative stress, accompanied by upregulation of the unfolded protein response and the protein ubiquitin pathways (Gião *et al.*, 2022). Oxidative stress is likely caused by the accumulation of Aβ, tau and lipofuscin deposits (Gião *et al.*, 2022; Municio *et al.*, 2023; Saunders *et al.*, 2023).

AD is also characterised by chronic low-grade neuroinflammation, accompanied by an elevated expression of many genes associated with the acute phase response, cytokines and cell adhesion molecules in the CP cells. This is at least partially caused by a failure in immune cell recruitment processes by the CPs. (Gião *et al.*, 2022)

4.2.2 Parkinson's disease

The involvement of the CPs in Parkinson's disease progression is far less documented than it is in AD. However, given that PD is also characterised by the accumulation of neuropeptides, mainly alpha-synuclein, and impaired glymphatic function in clearing these peptides from the CNS, impairments in CP function likely contribute to progression of PD. The CPs have been seen to contribute to the differentiation of dopaminergic neurons, whose degeneration is characteristic of PD, and recently, newer studies have also linked volumetric changes in the CP to the severity of PD symptoms (Jeong *et al.*, 2023; He *et al.*, 2024). A study by He et al. showed patients with enlarged CP volume experienced more rapid cognitive decline than those with a normal CP volume (He *et al.*, 2024). They stated that CP enlargement during early-onset PD could predict a faster cognitive decline and there was evidence that this change might be mediated by the Aβ/alpha-synuclein protein ratio in CSF (He *et al.*, 2024). Another study by Jeong et al. showed severe motor deficits and a decrease in striatal dopamine transporter availability correlated with enlarged lateral plexuses, suggesting that an enlarged lateral CP could serve as a biomarker for the disease. (Jeong *et al.*, 2023). However, more research on the role of CP and BCSFB in PD is needed.

4.3 Choroid plexuses in neuropsychiatric diseases

Alterations in the CP-BCSFB system during development have been documented in several neuropsychiatric disorders, including schizophrenia, psychosis, autism spectrum disorder, bipolar disorder and depressive disorder (Demeestere *et al.*, 2015; Lizano *et al.*, 2019; Zhou *et al.*, 2020). Alterations in the BCSFB are also present in chronic stress and sleep deprivation, which are significant risk factors for many psychiatric disorders (Demeestere *et al.*, 2015).

4.3.1 Schizophrenia and other psychosis disorders

Schizophrenia is a neuropsychiatric disease characterised by symptoms of psychosis, including delusions, hallucinations and disorientation in one's behaviour. It is believed to develop as a result of interactions via multiple genetic and environmental factors and is often accompanied by other psychiatric and non-psychiatric diseases, such as anxiety, depression, cardiovascular disease and metabolic diseases (Demeestere *et al.*, 2015; Zhou *et al.*, 2020). The cardiovascular disease and

metabolic diseases often arise as side-effects of antipsychotic medications, and they cause the life expectancy in schizophrenia to be decreased by approximately 15-20 years (Zhou *et al.*, 2020).

Abnormalities of the choroid plexuses in schizophrenia were first discussed a century ago, in 1921, when the CPs of schizophrenia patients were documented to have longer and smaller CPE cells, degeneration of the vascular endothelium, cellular fat depositions and large cystic formations. Around the time, individuals with bipolar mania were also identified with hypersecretion of the CSF and degenerations of CP connective tissue and vascular endothelium. Therefore, although psychosis symptoms and their connection to the CPs are often studied with cases of schizophrenia, the CPs have been documented to play a part in psychosis symptoms across several disorders. A straightforward example of the involvement of the CPs in psychosis symptoms is the development of third and fourth ventricle choroid plexus papilloma, which in some cases have caused symptoms of psychosis alongside depressiveness and impaired cognition with full symptom remission after papilloma clearance. Although the cases of neurodevelopmental disorders like schizophrenia are not as straightforward, the direct link between psychosis symptoms and CP abnormalities in the third and fourth ventricular CP papilloma is interesting. (Lizano *et al.*, 2019)

Fairly recently, volumetric changes in both the ventricular spaces and the CPs themselves have been shown to correlate with psychosis symptoms (Demeestere *et al.*, 2015; Lizano *et al.*, 2019; Zhou *et al.*, 2020). Increased volumes of the ventricular spaces in psychosis may be linked to a dysfunction in CSF secretion. This suggestion is supported by the fact that the gene responsible for the expression of the AQP1 water channel is located in the genetic loci that are strongly associated with schizophrenia (Demeestere *et al.*, 2015). The lateral ventricular CPs themselves have been seen to significantly increase in volume in individuals within the psychosis spectrum (Lizano *et al.*, 2019). This seems to be a hereditary feature, as the intermediate enlargement of the lateral CPs in comparison to controls has been documented in the healthy first-degree relatives of psychosis patients (Lizano *et al.*, 2019).

4.3.2 Chronic neuroinflammation in psychosis and depression

Chronic low-grade inflammation has also been documented in psychosis, which connects to alterations in CP function. In several studies of psychosis patients, chronic low-grade inflammation has been identified by increased inflammatory cell counts in brain tissue, elevated immunoglobulin G antibody levels in the CSF, increased ICAM1 expression in the CPs and elevated interleukin levels in blood circulation (Demeestere *et al.*, 2015; Lizano *et al.*, 2019). In a transcriptome sequencing study by Kim et al., an upregulated co-expression of genes related to immune function and inflammation was shown in the CP of schizophrenia patients. This transcriptional upregulation

was associated with disease status, and positively correlated with elevated levels of cortisol, pro-inflammatory cytokines and C-reactive protein in the serum and frontal cortex. Kim et al. suggested that the activation of schizophrenia might be triggered by a peripheral viral or bacterial stimulus. They might activate the immune and inflammatory genes in the CP, which would be accompanied by genetic susceptibility for developing the disease (Kim *et al.*, 2016).

The CPs play a role in the regulation of inflammatory, stress, neurotrophic and neuroendocrinological processes, which mediate allostatic load in schizophrenia. The term “allostatic load” refers to the cumulative “wear and tear” on the body as a result of chronic or repeated stress. It has been suggested that the CPs are overly active in schizophrenia because they try to regulate the increased allostatic load, which results in larger plexus volumes. Psychosis itself has also been deemed as biologically “toxic” to the brain, possibly increasing neuroinflammation. (Zhou *et al.*, 2020)

An upregulation of neuroinflammatory pathways has also been documented in depression patients, with increased pro-inflammatory cytokine, acute phase protein, chemokine, adhesion molecule and macrophage levels, as well as an upregulation of several genes involved in the neuroinflammatory response (Demeestere *et al.*, 2015; Althubaity *et al.*, 2021). Like in psychosis disorders, the chronic neuroinflammatory response in depressive disorders has been seen to correlate with a significant increase in CP volume compared to control individuals (Althubaity *et al.*, 2021). Studies with depressed patients have also documented a downregulation of cytoskeletal and extracellular matrix proteins, which may result in compromised BCSFB function (Demeestere *et al.*, 2015). Considering how CP enlargement is documented in psychiatric diseases, chronic stress, mild cognitive impairments and Alzheimer’s disease, further studies on the enlargement of the CPs and associated processes are greatly needed (Demeestere *et al.*, 2015; Lizano *et al.*, 2019; Althubaity *et al.*, 2021).

5. DRUG DELIVERY ACROSS THE BLOOD-CEREBROSPINAL FLUID BARRIER

The two brain barriers, the BBB and the BCSFB, make developing efficient drug treatments for neurological diseases difficult, because they restrict the free passage of solutes into the CNS. The lack of understanding of the brain barriers and the biological mechanisms behind neurological

diseases has resulted in a scarcity of effective treatments. It has been estimated that a large number of drugs for neurological diseases currently in use are ineffective, due to the ignorance of the brain barriers when developing the drugs *in vitro* (Saunders *et al.*, 2023). Some novel mechanisms to surpass the BBB barrier have been discovered, including specific carrier protein findings, as well as technologies to temporarily open the route across the BBB via osmotic disruptions and ultrasound technologies (Strazielle and Ghersi-Egea, 2016). The BBB, which is formed by the endothelial cells of the brain capillaries and their tight junctions, has received significantly more research attention than the BCSFB in drug delivery methods (Saunders *et al.*, 2023). The BCSFB, made of the tight junctions connecting the CPE cells, is less tight than the BBB and therefore acts as a route for solutes to bypass the BBB and enter the CNS (Praetorius and Damkier, 2017).

In a thorough review article in 2016, Strazielle and Ghersi-Egea collected the potential pathways to exploit for drug delivery purposes across the BCSFB (Strazielle and Ghersi-Egea, 2016). They stated that the research to surpass the brain barriers has underestimated the potential of drug delivery across the BCSFB (Strazielle and Ghersi-Egea, 2016). Regarding solute transport via passive diffusion across the BCSFB, they pointed out that the transport of most small-molecule drug candidates is limited by several types of solute carrier transporters at the CPE cell membranes (Strazielle and Ghersi-Egea, 2016). Relatively large molecules up to 12 nm can pass through the porous capillary endothelium in CP, but the further transport across the CPE cells would have to occur either via carrier-mediated influx pathways or transcytosis pathways, with the transcytosis pathways holding the most potential at the moment (Strazielle and Ghersi-Egea, 2016). Paracellular transport, which is the transport of molecules between the cells rather than through, is limited via the tight junctions connecting the CPE cells (Praetorius and Damkier, 2017).

5.1 Transcytosis pathways

The CPE cells display an active and abundant vesicular transport system via clathrin-coated pits, caveolae and fluid-phase endocytosis, which are the most common ways of endocytosis (Praetorius and Damkier, 2017). Transcytosis pathways could be used to transport drugs from the circulation into the central nervous system by tagging or attaching drugs to solutes or their fragments, which are naturally transported across the brain barriers (Strazielle and Ghersi-Egea, 2016; Saunders *et al.*, 2023). The transcytosis pathways that have been suggested as possible to utilise in drug delivery systems are receptor or carrier protein meditated and can be divided into pathways that are common for both the BCSFB and BBB, and pathways that are specific to the BCSFB (Strazielle and Ghersi-Egea, 2016). The transcytosis pathways that are applicable for drug delivery research for both BCSFB and BBB include a transferrin receptor pathway (TfR1) in clathrin-coated pits, the insulin receptor pathway, the low-density lipoprotein (LDL) receptor pathway and the LDL receptor-related protein family (Strazielle and Ghersi-Egea, 2016). Transcytosis pathways that

could be used in carrying drugs specifically across the BCSFB are the folate pathway and plasma protein transport, where folate and plasma proteins like albumin would be tagged with drugs, respectively (Strazielle and Ghersi-Egea, 2016). It needs to be stated that the solute transport pathways need extensive further research, and some pathways are more well-known than others. It is also not known how the tagging of a drug would affect the carrier's natural transcytosis pathways.

5.1.1 Mechanisms common to the BBB and the BCSFB

In the TfR1 pathway, TfR1 receptors in the clathrin-coated pits of the cell membrane bind iron-loaded transferrin. The complex moves inside the cell by endocytosis. The receptor is recycled back to the cell membrane and iron is exported into the cytosol for intracellular use in metabolic functions. To deliver iron to the brain via the TfR1 pathway, it needs to be exported from the brain-facing membranes of BBB or BCSFB cells with mechanisms involving ferroportin, vesicular transport, or some other currently unknown mechanism. (Strazielle and Ghersi-Egea, 2016)

Both the BBB and BCSFB possess a high insulin-binding capacity and the insulin movement across the barriers occurs via receptor-mediated transcytosis. The abundance of insulin receptors in the choroidal epithelium is promising in assessing the pathway for drug delivery, but the directionality of the transcytosis process and its characteristics must be further determined. (Strazielle and Ghersi-Egea, 2016)

The LDL receptor is involved in cell membrane cholesterol homeostasis, binding cholesterol-carrying LDL molecules and transporting them into the cell by endocytosis. The receptor is known to be expressed in abundance in the BBB, but its expression at the BCSFB and the specifics of its transcytosis mechanisms are lesser known. More research needs to be conducted in order to evaluate the receptor's drug delivery potential. The LDL-receptor-related protein family (LRP), on the other hand, has a few potential candidates for cerebral drug delivery. LRPs are structurally related to the LDL receptor and bind a large variety of ligands, including protease/protease inhibitor complexes and loaded carrier proteins for vitamins and hormones. (Strazielle and Ghersi-Egea, 2016)

5.1.2 Mechanisms specific to the BCSFB

Folate is a B9 vitamin that functions as a cofactor in basic metabolic reactions. It is present in the CSF and cotransported through the CPE cells with a folate receptor. Folate is taken into the CPE cells via endocytosis, transported by endosomes, and released into the CSF inside exosomal vesicles. Cancer cells often overexpress folate receptors and therefore folate-conjugated anti-cancer drugs could effectively be transported into the CSF to target tumours. (Strazielle and Ghersi-Egea, 2016)

Plasma protein transport happens through CPE cells, and although the exact mechanisms of plasma protein transport need to be further determined, albumin family proteins have been proposed as carriers for drug delivery (Strazielle and Ghersi-Egea, 2016; Saunders *et al.*, 2023). The CPE cells express several albumin-binding proteins, which could potentially transport albumin-bound molecules across the CPE cells. These proteins include SPARC (secreted protein acidic and rich in cysteine), glycophorin A and glycophorin C (Strazielle and Ghersi-Egea, 2016). Thus, it has been proposed that synthesising a peptide of albumin could serve as a vector to which drugs could be attached and carried across the BCSFB (Saunders *et al.*, 2023). However, further research is still needed. The albumin transport methods across the CPE cells vary between species, and the molecular structures of many albumin family members are unknown (Saunders *et al.*, 2023).

These briefly introduced transport methods hold some potential for reaching the central nervous system without physically disrupting the BBB. These drug delivery mechanics across the BCSFB could work alongside drug delivery mechanics across the BBB to enhance therapeutical responses (Strazielle and Ghersi-Egea, 2016). Every proposed drug delivery method listed above, as well as the ones discussed outside of this thesis, needs further research that also takes into account environmental factors, such as the fluid mechanics of CSF flow and how it carries the potential therapeutic molecules (Saunders *et al.*, 2023). A lot is still unknown regarding the mechanics of the brain barriers and how to therapeutically target the CNS, but with a better understanding of the physiological processes involved, drug delivery across the BCSFB is a promising approach to treating CNS disorders.

6. CHOROID PLEXUSES IN AGEING

The morphological structures of the choroid plexuses degrade with healthy ageing. The CPE cells flatten with an 11% loss in cell height at 88 years of age, consequently shrinking in overall volume (Damkier *et al.*, 2013). The length of the apical microvilli on the CPE cells also decreases (Marques *et al.*, 2017). The endothelial blood vessel walls in the CPs thicken, and the ventricular spaces around the plexuses expand (Marques *et al.*, 2017). CP function declines, which is seen as lowered expression of key proteins, such as AQP1 and carbonic anhydrase II (Marques *et al.*, 2017). The overall secretion of the CSF decreases with ageing, as does the overall CSF turnover rate. The resulting disruption to the CSF homeostasis can play a part in the development of neurodegenerative diseases as the waste-clearance system deteriorates.

Inside the CPE cells, intracellular inclusions of lipid byproducts and protein accumulations appear in healthy ageing, although significantly less than in diseased states (Damkier *et al.*, 2013; Gião *et al.*, 2022). The increase in the number of these inclusions continues until the age of 70, when it reaches a plateau (Damkier *et al.*, 2013).

As has been documented in multiple diseases, ahronic neuroinflammation is also present in healthy aged brains, with an induced type I interferon and a reduced type II interferon response in the choroid plexuses of aged humans and mice. Experimental repair by blocking type I interferon signalling in aged mice has been seen to partially restore cognitive function that had been lost due to ageing. (Baruch *et al.*, 2014) In addition, at least the lateral plexuses have been seen to expand in volume as they age in a healthy manner (Alisch *et al.*, 2021). Significant changes in the CP volume have also been associated with neurodegenerative and -psychiatric diseases with neuroinflammation present (Alisch *et al.*, 2021; Althubaity *et al.*, 2021).

One of the common pathological changes affecting the CPs in old age is stromal fibrosis, which is the thickening of the CP connective tissue (Prineas *et al.*, 2016). The increased amount of connective tissue forms nodules, which may calcify (Prineas *et al.*, 2016). The calcification of the CPs as well as other brain structures is documented in healthy ageing, but it might also be involved in neurodegenerative or -psychiatric processes (Junemann *et al.*, 2023). The solid formations found in postmortem human CPs that exhibit calcification are layered with calcified and noncalcified layers (Junemann *et al.*, 2023).

The age-related modifications in healthy choroid plexuses are still not thoroughly documented. Many of the morphological changes seen in diseased plexuses do occur in the CPs of healthy aged individuals as well. The line between a healthy aged CP modification and a pathological one is hard to determine because of the large number of changes in the CP tissue that occur throughout one’s life (Prineas *et al.*, 2016). It would therefore be important to further study the modifications in healthy and diseased human choroid plexuses of all ages, to specify which modifications can be classified as pathological. This project was undertaken to determine which of the available hippocampal region samples contained a lateral choroid plexus. The future aim is to further investigate the age-related changes occurring in CP tissue.

7. MATERIALS AND METHODS

Previously collected and stained histology samples of the hippocampal region from the Tampere Sudden Death Study (TSDS) were evaluated under a microscope to identify the presence of choroid plexus. The Tampere Sudden Death Study includes a collection of six individual brain regions (frontal cortex, insula-putamen, hippocampus, substantia nigra, pons and cerebellar cortex) from around 600 post-mortem cases aged 16-95 years fixed and stored in paraffin. The samples were collected during the years 2010-2015 and include post-mortem interval- and age criteria matching sudden deaths of individuals that died outside of hospital institutions in Tampere and the surrounding areas during that period. The samples and their use have ethical and research permission from the relevant authorities.

601 hippocampus samples available for the project were examined under a microscope to determine the possible presence of lateral ventricle CP tissue.

The samples were previously stained with haematoxylin and eosin (H&E). Haematoxylin stains the cell nuclei purple, and eosin colours the extracellular matrix and the cytoplasm pink. The staining protocol is widely used in histology, for it is quick and inexpensive, and can be used to diagnose several histopathological modifications by revealing the structural components of a sample tissue.

8. RESULTS

Out of the 601 samples examined, 207 had a clear view of the lateral choroid plexus in the hippocampus section. See Figure 3 for an example of the lateral CP location in a hippocampus sample. In 85 samples, a separate paraffin-embedded tissue block was available of the third ventricle choroid plexus, resulting in 38 cases having both third and lateral ventricular CP. See Table 1 and Figures 4 and 5 for the characteristics of cases with CP according to age and gender. The case ages are divided into groups for simplicity.

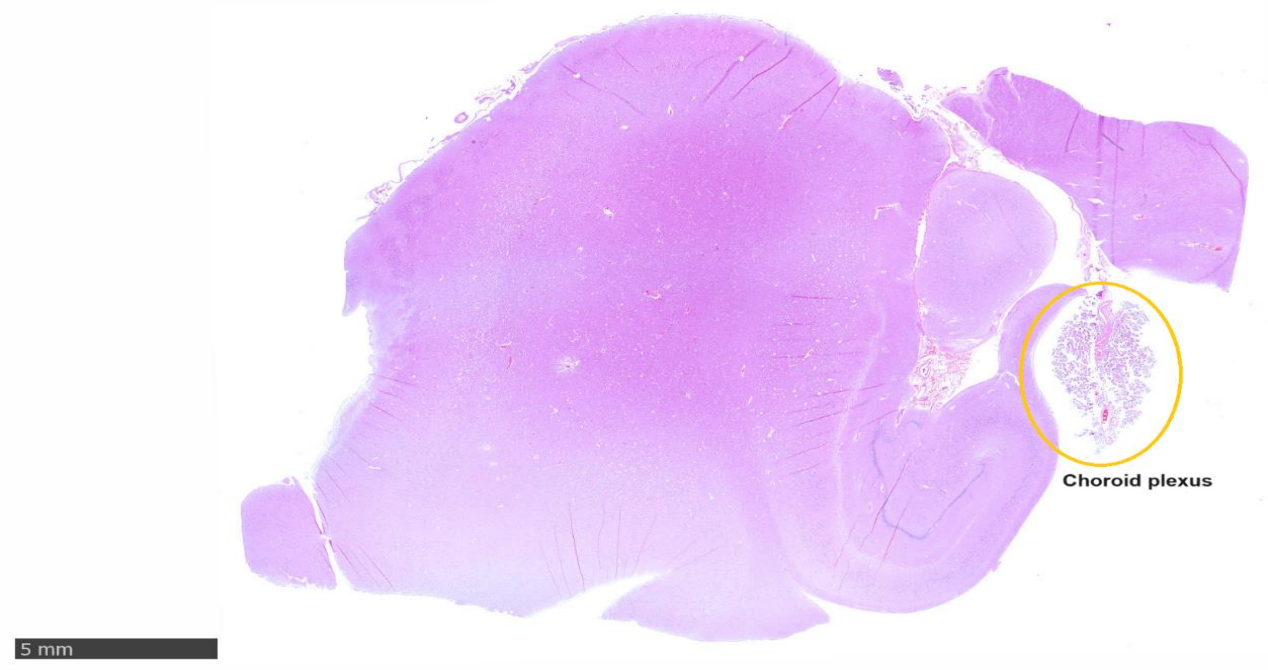

Figure 3: *A H&E-stained histological hippocampus sample. The lateral ventricle choroid plexus is present (see yellow circle).*

Table 1*: The number of cases that included a clear view of the choroid plexus, distributed by age groups and biological sex.*

Sex	Age group	plexus in lateral ventricle	plexus in 3rd ventricle	both plexuses	total
F	0-19	1	0	0	1
	20-29	2	0	0	2
	30-39	3	1	1	4
	40-49	4	2	2	6
	50-59	5	2	1	7
	60-69	14	3	2	17
	70-79	10	1	1	11
	80-89	18	6	2	24
	90+	2	1	0	3
	total (F)	59	16	9	75
M	0-19	5	0	0	5
	20-29	6	3	2	9
	30-39	15	4	2	19
	40-49	35	6	3	41
	50-59	39	15	4	54
	60-69	26	16	7	42
	70-79	21	16	6	37
	80-89	1	9	5	10
	90+	0	0	0	0
	total (M)	148	69	29	217
	total (F+M)	207	85	38	292

F = Female, M = Male

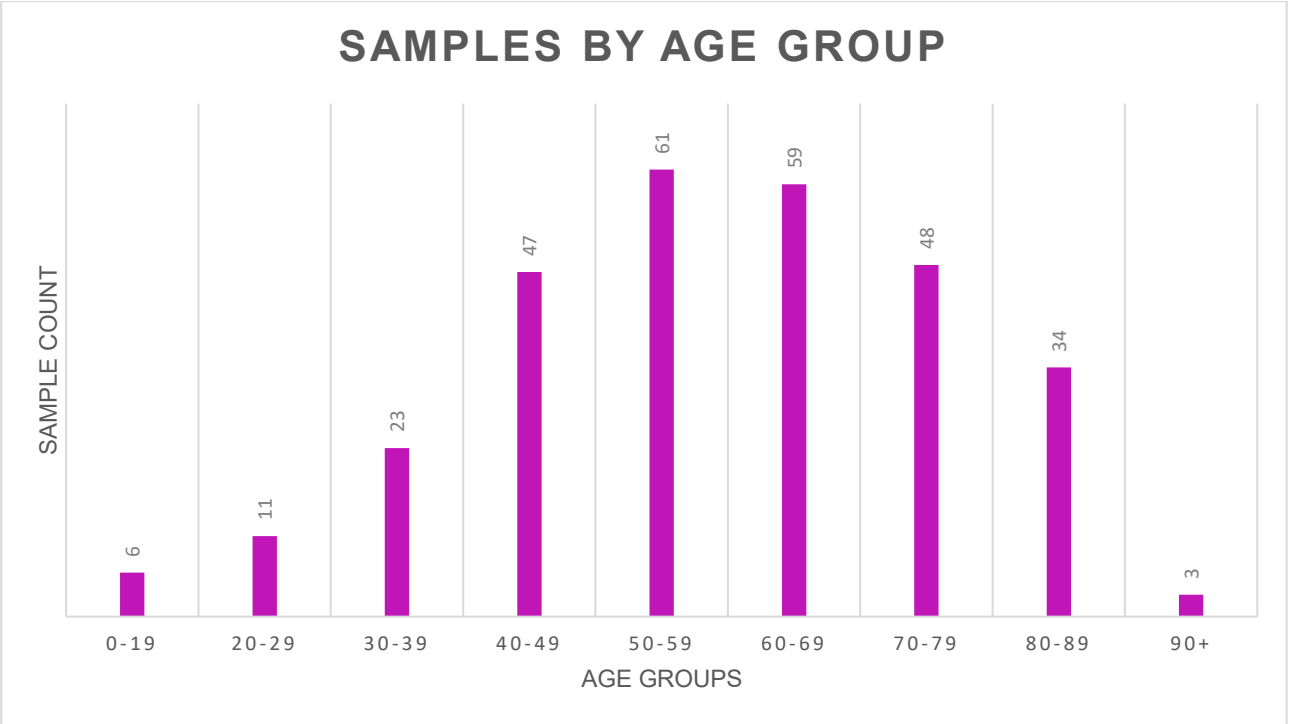

Figure 4: *Age distribution of the samples with choroid plexus.*

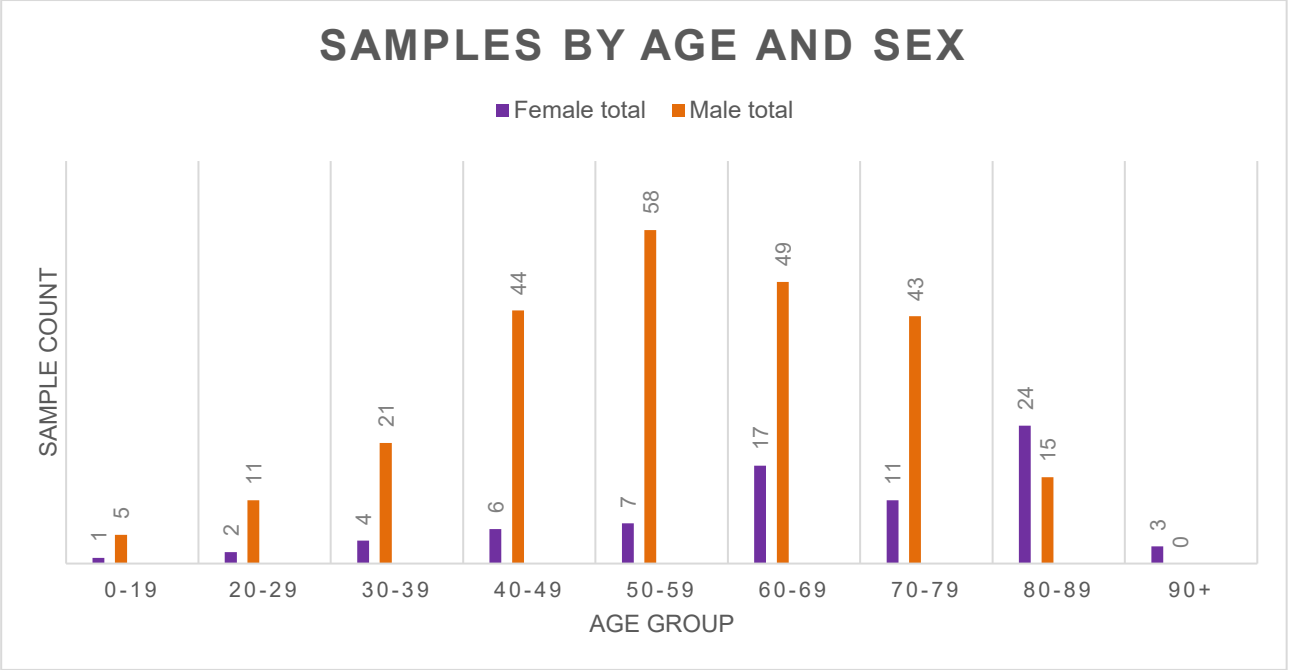

Figure 5: *Age and biological-sex distribution of the samples with choroid plexus.*

Some cases appeared healthy and others with fibrosis and calcification, although it was not the focus of this project to assess or measure this. An example of the fibrosis and calcification can be seen when comparing young CP tissue in Figure 6a and older CP tissue in Figure 6b. The results show we have a good range of samples across the adult lifespan in order to investigate the ageing process in the CP.

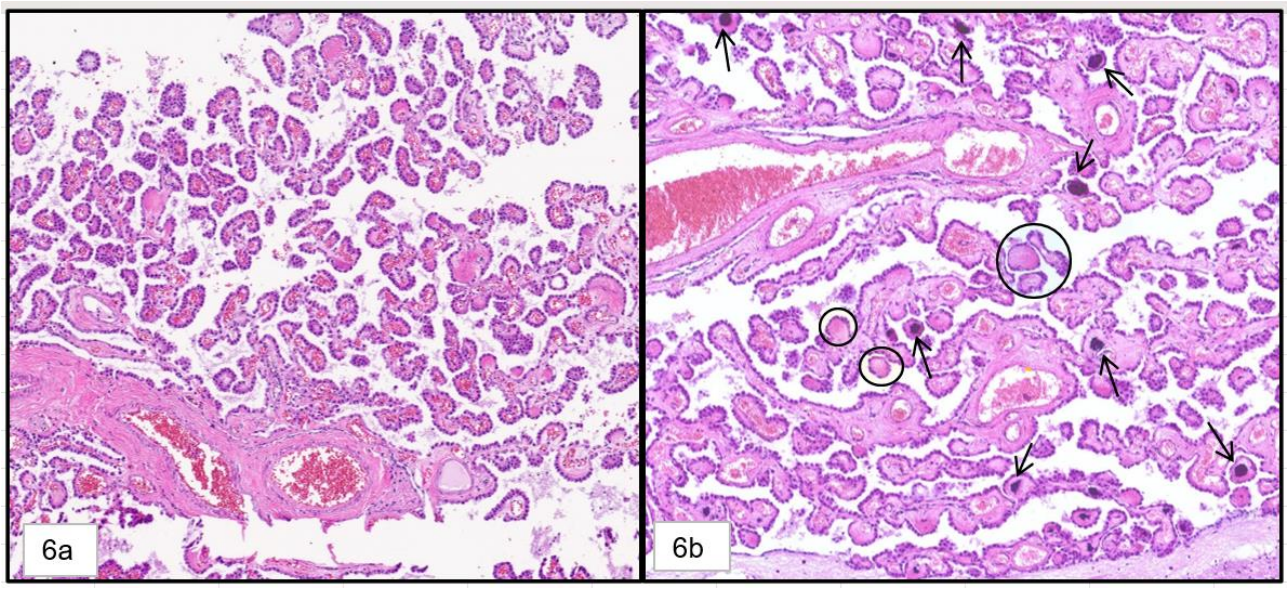

Figure 6a and 6b: *H&E-stained histological samples of lateral choroid plexuses from a younger (6a) and an older (6b) case. In 6b, examples of fibrosis of the connective tissue are circled. Arrows show visible calcification.*

9. DISCUSSION

As the results show, the study population has a range of ages (16 to 91 years) where plexus samples are available. The Tampere Sudden Death Study cases are notable in their range across all ages with several sample types, which can be used to assess the changes that occur in the CP during both healthy and diseased ageing. Although this project didn't focus on measuring pathological markers, in Figure 6b the effects of ageing in terms of fibrosis and calcification of the connective tissue can be seen. Figure 6a from a younger individual, is an example where fibrosis of the connective tissue or calcification is not present. In Figure 6b, a visible increase in the purple nodules indicates fibrosis, an amplified proliferation of connective tissue. The dark-purple nodules signify calcified fibrotic nodules, which may play a part in multiple disease pathologies (Junemann *et al.*, 2023).

With the obvious invasiveness in acquiring human CP tissue, a large amount of research on the CPs has been done using rodents and disease models. Although it is necessary to study rodent models in terms of practicality and ethics, it is important to note that the brains of rodents and humans are anatomically very different. The cerebral metabolic rate of rodents is twice as high compared to humans, and their CSF secretion rate is significantly larger, with the CSF turnover rate as high as 11 volumes/day (Benveniste *et al.*, 2018). Rodent disease models have been used

to study diseases like Alzheimer's disease and schizophrenia, although they lack exact comparability to the disease progression in humans (Saunders *et al.*, 2023). Oftentimes, the review articles of CP research do not specify, whether the information has been gathered with human tissue samples, cell culture models, or animal models, even though the results may vary significantly depending on the sample type. Thus, more transparency regarding the use of disease models in the literature is needed, and it is essential to invest in research that uses human samples in the future.

It has been noted only recently that most papers on the CPs do not specify which of the four plexuses has been studied, and when it is, it has often been the lateral plexuses. Although the plexuses of each ventricle share microscopic structure, the macroscopic traits and their environments are not the same. During embryonic development, the different plexuses develop separately and with variations in timing, progression, and developmental steps. It would therefore be important to focus on separating the plexuses in the literature and to include more research on the third- and fourth ventricle plexuses as well. (Saunders *et al.*, 2023)

9.1 Future prospects:

As has been discussed previously, the physiological effects of healthy and diseased ageing of the choroid plexus have not been thoroughly investigated, let alone on human tissue samples. Future research can be executed using the TSDS sample set with a total of 292 CP samples across all age groups and biological sexes to measure fibrosis and calcification in the CP tissue, in order to gain understanding of the effects that ageing has on the choroid plexus. It will be interesting to examine at what point CP fibrosis and calcification begin, and what is their cause or purpose.

The TSDS sample set can also be used to evaluate the inflammatory markers present in the CP tissue and tie this to the fibrosis and calcification modifications. As has been discussed previously in this thesis, the plexuses are key players in neuroinflammation and leukocyte recruitment into CNS inflammatory diseases. Chronic low-grade neuroinflammation has also been documented in multiple brain disorders, including Alzheimer's disease, psychosis and depression. Assessments of CP volume in correlation with chronic neuroinflammation could also be a topic of interest for future research.

Measurements of CP volume, fibrosis and calcification, and the connection with inflammatory markers, highlighting the ages at which they begin to accumulate and at what point and how they become degenerative in nature, would be important to determine to gain an understanding of their role in disease mechanisms.

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