Summary

Alzheimer’s disease (AD) is the most common form of dementia, currently estimated to afflict more than 35 million people worldwide. This neurodegenerative disorder is known to affect brain integrity and functioning, resulting in a deterioration of cognitive capabilities, including social cognition, memory, and personality changes. These changes lead to a significant loss in quality of life already at the early stages of the disease. At a cellular level, AD is characterized by progressive neuronal dysfunction and eventually loss of neurons, causing cognitive decline. The neuronal damage in AD is related to the deposition of extracellular amyloid β (Aβ) protein (plaques) and the intracellular aggregation of hyperphosphorylated tau protein. However, increasing evidence indicates that neuroinflammation and blood-brain barrier (BBB) dysfunction are key pathological hallmarks of AD as well. Altered lipid production and signaling, predominantly of the classes of sphingolipids and (oxy)sterols, might be a common denominator linking neuroinflammation and BBB dysfunction in AD. Therefore, the current thesis aims to define how changes in the sphingolipid balance and the responsible enzymes are associated with Alzheimer’s disease pathology. Moreover, our research also provides data on the role of nuclear liver X receptors in the inflammatory processes at the BBB.

Chapter 2 and 3 are pathological studies investigating the sphingolipid balance in different neurodegenerative diseases using post-mortem brain tissue. Sphingolipids are highly enriched in the brain and are essential for the development and maintenance of the functional integrity of the nervous system. Sphingolipid metabolism consists of a complex network of highly regulated pathways producing bioactive lipids that include sphingomyelin, ceramide, sphingosine, and sphingosine 1-phosphate (S1P). In most cell types, ceramide and S1P exert opposite effects on cell survival, where primarily ceramide is implicated in promoting cellular stress and cell death. Chapter 2 examines the sphingolipid profile of AD patients with capillary cerebral amyloid angiopathy (capCAA) compared to AD patients without capCAA. Since capCAA is implicated in promoting neuroinflammation in AD, we investigated sphingolipids and related enzymes that are linked to the neuroinflammatory response. Our results show an accumulation of ceramide in astrocytes whereas microglia show increased expression of the ceramide producing enzyme acid sphingomyelinase in capCAA cases. Furthermore, the activated glia cells express an increased number of S1P receptors. Overall, the brains of AD patients with capCAA show increased levels of long-chain ceramides while very long-chain ceramides are decreased, suggesting a shift towards a pro-apoptotic environment. Finally, the levels of S1P are elevated in AD patients with capCAA, which might be a protective counteracting mechanism to the high levels of pro-apoptotic ceramide. As a continuation on chapter 2, chapter 3 introduces the hypothesis that increased astrocytic ceramide content is a common denominator of neuroinflammation in general. We selected brain tissue of different dementia subtypes that differ in their neuroinflammatory status. The brain homogenates of the distinct dementia subtypes show increased pro-apoptotic ceramide, similar to what we found in AD with capCAA cases. Using a descriptive approach, we show that (again) reactive astrocytes increase their ceramide content in relation to the amount of neuroinflammation. In addition, investigation of the ceramide generating enzymes revealed that ceramide synthase 5 is a possible initiator of the increased ceramide content in astrocytes, further strengthening the hypothesis that astrocytes are responsible for pro-apoptotic ceramide production.
The neuroinflammatory environment that characterizes AD propagates chronic impaired function of the BBB. The BBB is indispensable for the maintenance of brain homeostasis and proper neuronal functioning. It is composed of specialized endothelial cells, which form a physical barrier between the blood and the brain. The highly differentiated brain endothelial cells are sealed together by means of tight junctions, thereby limiting paracellular exchange of solutes between cells. In addition, brain endothelial cells express specialized transporters which allows them to tightly regulate the bidirectional transcellular transport of molecules. This way, the BBB protects the central nervous system from injury and disease. Liver X receptors (LXRs) might provide the link between decreasing brain inflammation and exerting protective effects on BBB function. LXRs belong to a large family of nuclear receptors which upon activation stimulate gene transcription. Two LXR isoforms exist in mammals, termed LXR$\alpha$ (NR1H3) and LXR$\beta$ (NR1H2), which share over 75% amino acid sequence identity. LXRs play an important role in cholesterol and lipid metabolism. The best described process involving LXR function is reverse cholesterol transport where LXRs facilitate the elimination of excess cholesterol in response to cholesterol precursors or oxysterols. However, LXRs appear to be involved in a far broader spectrum of functions.

Chapter 4 provides a literature review of the LXRs as the possible link between neuroinflammation and blood-brain barrier dysfunction. We explore the function of LXRs that have been described in relation to the different cell types affecting the BBB, such as endothelial cells, pericytes and astrocytes. This overview demonstrates the anti-inflammatory effects that LXRs exert in various cell types. Moreover, we reveal the beneficial effects on the BBB after LXR activation and highlight gaps in our knowledge so far. One of these gaps is the lack of distinction made between the LXR$\alpha$ and the LXR$\beta$ isoform. Therefore, chapter 5 and 6 are directly focused on the separate role of LXRs in blood-brain barrier function. Chapter 5 shows the importance of LXR$\alpha$ in maintaining blood-brain barrier function under basal and neuroinflammatory conditions. In chapter 5 we investigate whether LXR$\alpha$ and LXR$\beta$ have different functions in the endothelial cells of the BBB. By performing in vitro experiments using endothelial cells, where we induced the knockdown of LXR$\alpha$ or LXR$\beta$, we show that mainly LXR$\alpha$ is important in maintaining BBB integrity. We further confirm our in vitro findings in vivo in an inflammatory mouse model. Under normal conditions, the specific knockout of endothelial LXR$\alpha$ does not affect the animal’s phenotype. However, under neuroinflammatory conditions, the mice lacking LXR$\alpha$ in their endothelial cells show higher disease score compared to control mice. Immunohistochemical analysis revealed a less tight barrier, increased expression of cell adhesion molecules by the endothelial cells, and a higher number of infiltrating cells in the spinal cord of the animals. Chapter 6 focusses on the mechanism underlying the role of LXR$\alpha$ in BBB (dys)function. Deep sequencing data of LXR$\alpha$ knockdown cells showed a difference in the adherence junction pathway compared to control endothelial cells. Specifically, we link LXR$\alpha$ to a process called endothelial-to-mesenchymal transition, where the reduced expression of LXR$\alpha$ results in the increase of SNAI2 upon which endothelial cells partly lose their phenotype and become more mesenchymal like. Moreover, the increased expression of SNAI2 in LXR$\alpha$ knockdown cells is related to a perturbed NOTCH signaling. Collectively, our findings suggest that LXR$\alpha$ is linked to the NOTCH signaling pathway via which BBB integrity is maintained.

Finally, chapter 7 discusses the experimental chapters and reflects on their overall contribution to knowledge about lipid signaling and metabolism in AD. The overarching aim
of this thesis was to investigate whether a deregulated sphingolipid pathway is critical in the neuroinflammatory process observed in AD. In addition, our research also provided data on the role of nuclear receptors in the inflammatory processes at the BBB. By increasing our insights in the pathological mechanisms underlying AD, i.e. neuroinflammation and BBB dysfunction, we may identify novel ways of treatment or effectively repurpose existing therapies for AD.