**English summary**

Microglia are the main resident immune cells of the central nervous system (CNS). Microglia have a small cell body and ramified branches that extend through the surrounding tissue and constantly survey their microenvironment for danger signals to maintain brain homeostasis. When microglia become “reactive” upon detection of danger signals, they alter their morphology from a highly ramified shape to a more ameboid shape with less branching dendrites and an enlarged soma, and release inflammatory cytokines and chemokines. One of the key functions of microglia is phagocytosis, the process of engulfing other cells or particles. Phagocytosis by microglia involves the clearance of apoptotic cells, cellular debris and pathogens, which is essential for maintaining tissue homeostasis. Besides their immune function, phagocytosis or remodeling of synapses by microglia, known as synaptic pruning, is a crucial process in brain development and plasticity. Synaptic pruning selectively removes weak or inactive synapses, thereby refining and strengthening synaptic connections during development and learning. Microglial function is tightly regulated by constant bidirectional communication with neighboring neurons and other cells to maintain an appropriate level of activity.

The dysregulation of microglial functions has been linked to aging and neurodegenerative diseases. During the aging process, the gene expression profile of microglia is altered, with downregulation of genes associated microglial homeostasis and upregulation of genes associated with immune response. With aging, microglia become less efficient at clearing debris and misfolded proteins, which are hallmarks of age-related neurodegenerative diseases, including Alzheimer's disease (AD), and leads to the accumulation of toxic aggregates and chronic inflammation of the brain. Although microglia respond to neurodegenerative insults and remove damaged cells and debris by phagocytosis under healthy conditions, chronic microglial activation can cause neuroinflammation by releasing pro-inflammatory cytokines, chemokines, and reactive oxygen species, which can further damage neurons and cause excessive synapse pruning, leading to neurodegeneration and disease progression.

In this thesis, the primary objective was to explore microglial phenotypes in various disease conditions and aging and determine how microglia acquire these disease phenotypes.

**Chapter 1** provides an overview and summarizes the current knowledge of function of microglia in healthy and disease conditions.
In chapter 2, we addressed the hypothesis that the perturbation of glial cell functions might reflect initial processes in AD etiology and associate with cognitive impairment. Here, we focused on determining the temporal onset of cognitive impairment and microgliosis in APPswe/PS1dE9 (APP/PS1) mice, a transgenic mouse model for increased amyloidosis for AD. We observed that gliosis, alterations of long-term potentiation (LTP), and cognitive deficits already occurred before the presence of hippocampal Aβ plaques in APP/PS1 mice. Furthermore, we examined the impact of minocycline, a tetracycline antibiotic, with various treatment timelines on the activation of microglia and its effect on AD-related disease progression. Treatment with minocycline to inhibit microgliosis resulted in a rescue of cognitive function and was most effective when started before the onset of microgliosis and when provided daily.

The microglia surrounding amyloid-beta (Aβ) plaques in the brains of AD patients undergo morphological changes characterized by reduced branching and enlarged cell soma. Furthermore, these microglia exhibit a distinct gene expression profile when compared to those in unaffected brain tissue, with an enrichment of genes associated with phagocytosis and lipid metabolism in the microglia associated with plaques. The subsets of microglia associated with amyloid plaques are called “disease-associated microglia (DAMs)”.

In chapter 3, we delineated the initial transcriptional changes in microglia in response to amyloid in APP/PS1 mice using both bulk and single cell RNA sequencing. Our aim was to determine the first transcriptional changes between homeostatic microglia and DAMs in order to identify early changes in microglia gene expression along amyloid pathologies. By bulk RNA-seq, transcriptomic changes were detected in hippocampal microglia from 6-months-old APP/PS1 mice. By performing single-cell RNA-seq of CD11c-positive and negative microglia from 6-month-old APP/PS1 mice and analysis of the transcriptional trajectory from homeostatic to CD11c-positive microglia, we identified a set of genes that potentially reflect the initial response of microglia to Aβ.

In aging and neurodegenerative disease conditions with chronic low-grade inflammatory stimulation, microglia can adopt a “primed” phenotype, characterized by an increased inflammatory state and an exaggerated response to inflammatory challenges. The transcriptomic profile of primed microglia has been reported to represent a common gene activity signature observed in different neurodegenerative disease- and aging mouse models. Primed microglia display an increased expression of genes associated with phagosome, lysosome, and antigen presentation, which are
also enriched in DAMs. **Chapter 4** aimed to examine how homeostatic microglia acquire a primed microglia transcriptomic signature in *Ercc1*-deficient mice, an accelerated aging mouse model, where microglia priming has been observed. By performing morphometric, transcriptomic, and chromatin accessibility analyses of microglia in *Ercc1*-deficient mice at young ages, we further characterized microglia priming in this model over time and identified the transcription factors that regulate the transcriptomic signature of primed microglia. We identified a set of genes that was already upregulated in microglia from 6 week-old mice, and these genes were even more induced in 12 week old *Ercc1*-deficient mice, suggesting this gene set is involved in microglia priming. Furthermore, we observed an increase in transcription factor *Irf7* and *Irf9* expression and an enrichment of their binding motifs in accessible chromatin in primed microglia. This was paralleled by a decrease in *Smad3* expression and a depletion of SMAD3 binding motifs in chromatin accessibility assays. Our data suggest that increased IRF7/IRF9 activity and reduced SMAD3 activity are required for microglia priming.

Radiotherapy, a cancer treatment with high-energy rays or particles to destroy cancer cells, is a common treatment for brain tumors. It is often applied together with surgery or chemotherapy to target brain tumors. While radiotherapy is beneficial to shrink the cancer and reduce symptoms, many patients experience irreversible and often progressive cognitive impairment that affects their quality of life after radiotherapy. Microglia are sensitive to radiation-induced damage, and radiation increases the number and activity of microglia. Furthermore, elimination of microglia can ameliorate radiation-induced cognitive deficits in mice, indicating regulation of neurogenesis and neuroinflammation by microglia has an important role in radiation-induced cognitive impairment. In **Chapter 5**, we aimed to determine the long-term molecular and functional state of microglia after brain irradiation. By conducting a transcriptional analysis of microglia in brain-irradiated rats with subsequent LPS stimulation, we revealed that radiation induces upregulation of microglia priming genes as well as exaggerated immune responses with a subsequent peripheral stimulus. Additionally, we provided evidence that radiation led to persistent microglia priming, which appears to be dose-dependent and remains unaltered by radiation dose fractionation. Moreover, we demonstrated that microglia priming may occur in the brain of glioblastoma (GBM) patients who have received radiotherapy treatment.

Finally, in **Chapter 6**, we discussed common transcriptomic profiles observed
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across various disease conditions and potential causes of microglia priming. Furthermore, we offered insights into future perspectives for modulating microglia priming.