SUMMARY

The brain consists of several highly specialized cell types functioning in close collaboration. Neurons are connected by synapses and are capable of generating excitatory or inhibitory signals through release of neurotransmitters. In the adult mammalian brain, glutamate is the primary excitatory neurotransmitter, whereas γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter. Neurotransmission and brain energy metabolism are closely linked. Neuronal signaling is energetically expensive and consumes up to 80% of the total energy expenditure of the brain. Apart from the neurons, the brain is also made up of glial cells. The most abundant glial cell type is the astrocyte, which is intimately associated with synapses and is essential for uptake of neurotransmitters from the synapse. In turn, astrocytes provide neurons with glutamine for replenishment of the glutamate and GABA pools. The essential roles of astrocytes in glutamate uptake and metabolism are well established. However, little effort has gone into functionally exploring the extent of cellular GABA metabolism in the brain.

Alzheimer’s disease (AD) is a complex neurodegenerative disease characterized by cerebral accumulation of amyloid-β (Aβ), leading to progressive loss of cognitive function and dementia. AD develops during a long preclinical phase, spanning decades, before dementia symptoms arise. A hallmark of AD development is a steady decline of brain glucose metabolism correlating with clinical impairment. AD is further characterized by imbalances between excitatory and inhibitory signaling. The underlying mechanisms behind the metabolic decline and signaling disturbances in AD remain unclear. Furthermore, how Aβ pathology functionally affects the metabolic interplay of neurons and astrocytes is not known.

The aims of this PhD thesis were to 1) characterize cellular brain GABA metabolism and 2) determine how Aβ pathology functionally affects neuronal and astrocytic energy and neurotransmitter metabolism using the transgene 5xFAD amyloid mouse model of AD.

Using isotopically enriched GABA ($^{15}$N & $^{13}$C) we demonstrate that acutely isolated brain slices have a large capacity for GABA uptake and metabolism. Cellular metabolism of GABA was utilized extensively for glutamine synthesis in astrocytes, which was substantiated by selective pharmacological inhibition of GABA uptake in neurons and astrocytes. Finally, GABA exposure did not stimulate energy metabolism of cultured astrocytes, but was able to support uncoupled respiration.

Applying isotopically enriched substrates and acute brain slices of female 5xFAD mice, we found severe dysfunctions of neuronal and astrocytic energy and neurotransmitter metabolism. In the
advancing stages of Aβ pathology, we show that dysfunctional astrocyte metabolism leads to diminished glutamine synthesis, which in turn hampers neuronal GABA synthesis. This was accompanied by an elevated neuronal capacity of glutamine metabolism. Interestingly, astrocyte GABA metabolism was also found to be reduced in the 5xFAD slices.

In the early phases of Aβ accumulation, corresponding to the preclinical phase of AD, we show complex region and cell specific metabolic adaptations in the male 5xFAD brain. Hyperactive neuronal signaling was observed in the hippocampus, coinciding with reduced astrocytic and neuronal energy metabolism. As observed in the female 5xFAD mice, the diminished astrocyte metabolism resulted in hampered synthesis of glutamine, again impairing neuronal GABA synthesis. Surprisingly, the 5xFAD cerebral cortex displayed a generally maintained, and in some instances even elevated, metabolic capacity, suggesting stronger metabolic impacts of early Aβ pathology on the hippocampus. This was in line with reduced metabolic function of isolated synaptosomes from the 5xFAD hippocampus.

The studies of this thesis demonstrate clear astrocyte involvement in cellular GABA metabolism. This knowledge was applied in the 5xFAD mouse as a model system of AD, showing clear impairments of particularly astrocyte energy and neurotransmitter metabolism. In the early stages of Aβ accumulation, complex regional dysfunctions of both neuronal and astrocytic energy metabolism were observed in the 5xFAD mice. Overall, the studies suggest that metabolic perturbations of astrocytes and disturbances of the neuron-astrocyte metabolic relationship could be fundamental for the synaptic dysfunctions of AD.