Microstructural and metabolic alterations in the zebrafish brain induced by toll-like receptor 2 deficiency: insights from ultra-high field magnetic resonance imaging and spectroscopy

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SUMMARY
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Microstructural and Metabolic Alterations in the Zebrafish Brain Induced by Toll-Like Receptor 2 Deficiency

Insights from Ultra-High Field Magnetic Resonance Imaging and Spectroscopy

Advanced UHF MRI techniques provide powerful tools for studying the zebrafish brain, an emerging model in neurological research. In this work, various MRI and localized MRS methods are optimized and employed for UHF strength for studying the zebrafish brain. Specifically, these techniques are utilized to study the effect of TLR2-deficiency. Toll-like receptors, especially TLR2, are vital for recognizing pathogens and initiating immune responses. There is strong evidence that TLR2 is involved in the pathology of several neurological disorders. While essential for fighting infections, its dysregulation can lead to neuroinflammation and neurodegeneration. The full extent of TLR2's role in normal brain function remains poorly understood, requiring further research. The current work shows advanced imaging techniques allow to monitor microstructural and metabolic changes in the brain of a TLR2 deficient zebrafish model. This approach provides unprecedented detail and accuracy in studying the effects of TLR2 deficiency on zebrafish brain structure and function.

In **Chapter 1**, of this thesis, I provide the context and theoretical background of the various MRI, diffusion-based MRI, and MRS techniques utilized in the research. This chapter lays the foundation by explaining the principles and applications of these imaging methods, detailing how they contribute to our study. Additionally, the scope of the thesis is outlined, highlighting the specific objectives and research questions addressed in the subsequent chapters.

Chapter 2, focuses on the application of UHF MRI at 28.2 T for the visualization of brain structures and white matter tracts in zebrafish. Longitudinal relaxation times T_1, and transverse relaxation times T_2, are estimated for their significance in method optimization and their dependency on the applied magnetic field strength $\mathbf{B}_0$, showing an increase in T_1 and a decrease in T_2 compared to lower field strengths. For the visualization of major brain structures, the RARE sequence is optimized, providing high resolution anatomical images at a resolution of 23 µm × 23 µm. Furthermore, additional contrast is obtained by the utilization of diffusion properties in brain tissue through the utilization of DWI. Additionally, DTI is optimized for the estimation of diffusivity metrics. For the visualization of white matter structures, dMRI tractography employing stTDI msmt CSD is utilized on zebrafish brain data. The first results of these methods for intact zebrafish, show the ability to visualize tiny white matter structures in the zebrafish brain.

In **Chapter 3**, MRI, dMRI, and dMRI tractography methods are utilized for monitoring microstructural changes in the brain of *tlr2*$^{-/-}$ adult zebrafish. Anatomical imaging by RARE sequence reveal significant changes in the *tlr2*$^{-/-}$ brain morphology, while MSME indicate increased T_2 at various brain regions. Mono- and multi component analysis of the DWI data reveals a decrease in the apparent diffusion coefficient, which contributed specifically to an increase in slow component. These results potentially indicate astrogliosis, cytotoxic edema or inflammatory processes in the brain of *tlr2*$^{-/-}$ zebrafish. Zooming in on white matter structures, DTI and DKI reveal significant decrease in diffusivity and increases in diffusivity kurtosis in multiple white matter structures, indicative of reduced white matter integrity or inflammatory processes. Intriguingly, a potential link to neurobehavioral changes observed in *tlr2*$^{-/-}$ models can be made, including diminished spontaneous activity, diminished food consumption and disrupted circadian rhythms. Our results show that UHF MRI, dMRI, and dMRI tractography provides the necessary resolution and SNR to study microstructural changes in tiny structures in the brain of pathological zebrafish models.

Chapter 4 focuses on the optimization of single voxel localized ^{1}H MRS to monitor the neurochemical composition of the zebrafish brain at 28.2 T. UHF MRS provides MR spectra from selected regions as small as 125 nL. Highly resolved spectra were obtained and allow for the direct (relative) quantification of several metabolites, which was not yet achieved at lower magnetic field strengths. Although superior SNR and spectral resolution can be obtained, MR artifacts, specifically chemical shift displacement effects, are pronounced at 28.2 T. This required the utilization of strong gradient pulses with broad transmitter RF pulse bandwidth and a finely tuned excitation frequency for the metabolites of interest. After optimization, stable metabolic profiles are obtained from various locations in the zebrafish brain. This allows for the monitoring of the neurochemical environment across different brain regions. By adjusting acquisition parameters and leveraging the high spectral resolution available at 28.2 T, it is possible to minimize artifacts and maximize the reliability of the metabolite quantification. Consequently, this enables precise and reproducible mapping of metabolic changes, which is crucial for studying the brain's biochemistry under various physiological and pathological conditions.

In **Chapter 5** HR-MAS spectroscopy is utilized to monitor neurochemical changes in the brain of TLR2-deficient zebrafish. These data showed significant changes in the levels of the neurotransmitters glutamate and GABA, glutamine, alanine, lactate and myo-inositol, indicating alterations in neurotransmission and metabolic processes potentially indicative of excitotoxicity, increased cerebral energy demand, and astrogliosis. Furthermore, localized MRS was utilized to provide the metabolic profile of specific brain regions in the zebrafish. By targeting distinct areas within the brain, this technique allowed to measure regional variations

in metabolites, offering a detailed view of metabolic changes associated with TLR2-deficiency. This region-specific metabolic profiling enhances understanding of how TLR2 impacts various brain functions and structures, shedding light on its role in neurodevelopmental and neurodegenerative processes. The forebrain, midbrain, and hindbrain show high levels of lactate, indicating a brain wide increase in energy demand as a consequence of TLR2 deficiency. Furthermore, decreased levels of NAA are observed in the hindbrain, in a region overlapping with important white matter tracts, indicating TLR2-deficiency potentially has an effect on white matter integrity.

Finally, in **Chapter 6**, the general conclusion and future outlook of this work is provided.