MANGANESE TRACT TRACING IN ZEBRAFISH

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Background

Manganese (Mn2+) is a metal that can enter excitable cells using Ca2+ transport systems, and can bind to a number of intracellular structures as it has high affinity for Ca2+ and Mg2+ binding sites. Paramagnetic forms of manganese ions are potent MRI relaxation agents, and can thus be used to track neuronal activation and pathways. This use of manganese-enhanced MRI (MEMRI) to visualize neural circuits in rodent animal models has rapidly increased in recent years.

Zebrafish have emerged as an invaluable vertebrate system for the study of genomics, development, human disease processes, and for drug discovery. Much of the initial success of zebrafish studies has been due to the transparency of the fish during embryogenesis and the use of the Green Fluorescent Protein reporter system to study localized gene expression and function in the transparent embryo. The use of adult zebrafish as an animal model for a diverse range of applications continues to rapidly expand, especially as adult zebrafish are able to regenerate damaged tissues including cardiac muscle and spinal cord. For this reason zebrafish biology is an area currently of great interest in the field of stem cell and regenerative biology. Understanding of how this occurs in the adult fish may offer insight into new therapies for human tissue repair in conditions such as in spinal cord injury and myocardial infarction. Compared to rodents, zebrafish are inexpensive to acquire and maintain, and just as with mice, animal models of human disease can be created by genetic and environmental manipulation. GFP cannot readily be used in vivo in adult fish due to lack of light penetration. The ability to be able to perform MEMRI in zebrafish models would introduce these models to a vast array of neuronal and cardiac studies that can currently only be performed in rodents.

Methods and Results

To perform MEMRI the animal has to be alive. Therefore we have designed and built a new zebrafish micro-imaging system which provides high resolution *in vivo* imaging: (Fig. A). The fish is held in silica glass tube around which the coil is secured. MS-222 is used to anesthetize the fish minimizing motion. Temperature–controlled, oxygenated water continuously flows over the gills during imaging using a pump. As the tube is only just wide enough to hold the fish, large amounts of “unused” water do not flow through the tube which minimizes signal loss from susceptibility and turbulence. Using a Bruker 7T small animal system we have optimized in vivo anatomical imaging (Fig.B), as well as 2D rare, 2D spin echo, and 3D flash pulse sequence protocols specifically suited for in vivo zebrafish micro-imaging imaging of Mn2+.

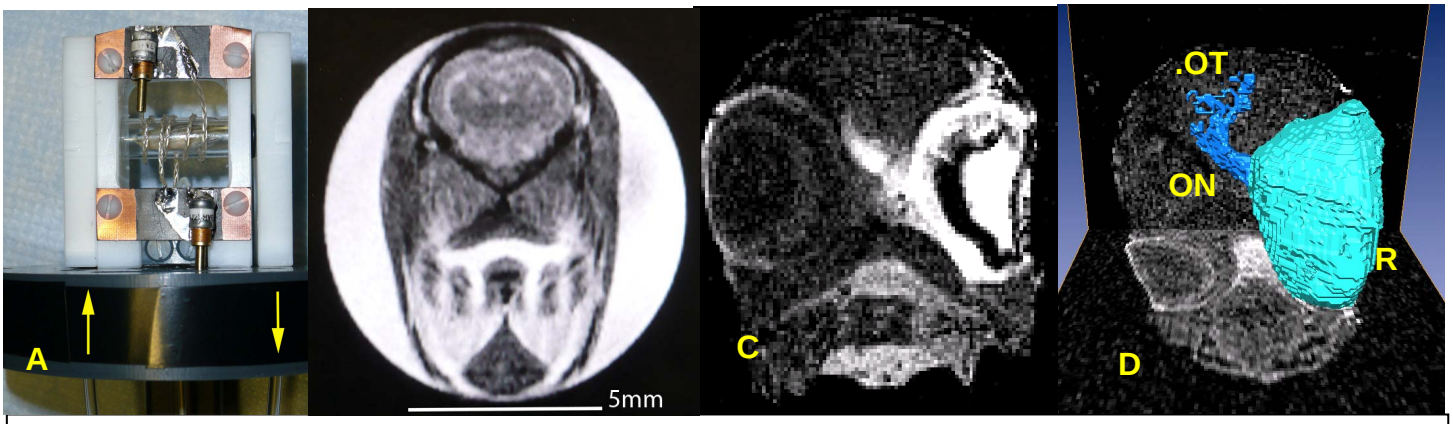

Fig. A *In vivo* coil and fish holder system for imaging anesthetized zebrafish provides continuous flow of water over fish. **Fig. B** Example of anatomical imaging using aT2 rare sequence. **Figs. C&D** 6 hrs after unilateral ocular injection of Mn. Mn appears as high intensity in the activated neurons, and outlines the retina (R) and optic nerve (ON) and optic tectum (OT). 3D segmentation and surface rendering of the high signal in the optic tract outlines the zebrafish visual system. Eye is colored blue-green, the optic tract is blue. The visual system in the fish is positioned anteriorly in the brain.

Discussion and conclusion

Using this system we demonstrate that in vivo MEMRI can be performed in this new animal model. We are able to demonstrate the different position of the visual cortex in the zebrafish as compared to humans. The excellent vision that zebrafish have as compared rodents may make these fish a better model for the study of diseases of the visual tract. MEMRI of course can be applied to other neural circuits as well, in addition to being used for extra neuronal studies such as in the heart.