Magnetic Resonance Microimaging of Adult Zebrafish Brain Using Cryoprobe Technology

A. Alia[1], S. Kabli[1], D. Gross[2], D. Marek[3], T. Oerther[2], M. Sacher[3], H. P. Spaink[4], and H. J. de Groot[1]

[1]SSNMR, Leiden Institute of Chemistry, Leiden University, Leiden, Leiden, Netherlands, [2]Bruker BioSpin GmbH, Karlsruhe, Germany, [3]Bruker BioSpin AG, Fallanden, Switzerland, [4]Institute of Biology, Leiden, Netherlands

Introduction

Due to similar organizations of brain components as that of human, zebrafish (*Danio rerio*) is increasingly used for understanding brain diseases including neurodegenerative disorders (1). However, investigating the brain of zebrafish in *vivo* are restricted to very early developmental stages due to opaqueness of the juvenile and the adult stages. Magnetic resonance microimaging (μMRI) is amenable to study optically opaque zebrafish brain non-invasively. However, in order to image such a small size of the brain with high resolution, it is essential to optimize the signal-to noise ratio. In this study we applied high resolution μMRI at 9.4T in conjunction with novel cryogenically cooled cryoprobes to improve SNR and to gain significant insight into the brain structures of adult zebrafish.

Methods

For imaging, adult zebrafish were euthanized by immersion in ice-cold water and subsequently fixed in 4% buffered paraformaldehyde. To study the brain structures, the head of the adult zebrafish were imaged at 9.4T using an vertical bore microimaging system (AVANCE spectrometer from Bruker BioSpin GmbH) equipped with Micro2.5 gradient system of 2.5 G/cm/A and with either conventional Micro2.5 probe with exchangeable 5 mm rf saddle coil or CryoProbe for microimaging. The CryoProbe is equipped with a ^{1}H channel for 5 mm diameter samples, an rf coil operated at a temperature of 25 K and an integrated cryogenic preamplifier operated at 77 K. The temperature of the cryogenic probe was fully controlled by the Bruker CryoPlatform. Cooling of the CryoProbes is accomplished with a closed-looped helium gas flow via a flexible transfer line. For brain imaging 2D and 3D Multi slice multi echo (MSME) sequence was adapted. SNR was measured from mean pixel intensities in rectangular ROIs drawn on the tissue and drawn on the background. Data acquisition and processing were performed with ParaVision3.02pl4 (Bruker BioSpin GmbH, Germany).

Results and Discussion

One area that stands to benefit from the zebrafish model is developmental neuroscience, and the challenges of understanding the detailed mechanisms underlying various brain disorders *in vivo* (2). Magnetic resonance microimaging in combination with cryoprobe technology offers a unique opportunity to study brain structure and function in zebrafish non invasively. In this study we applied novel cryoprobe technology at ultra high field strength for getting high resolution images from the brain of zebrafish using μMRI. Cryoprobe technology improves signal/noise (S/N) ratios by reducing the operating temperature of the coil and the pre-amplifier. As a result, the efficiency of the coil is improved and the noise of the coil and the pre-amplifier are reduced. The dramatic increase in the S/N ratio by a factor of 3-4, as compared to conventional probes (Fig. 1), leads to a reduction in experiment time of up to 16. Several structures within the brain are identifiable including the optic tectum, toris semicircularis, optic ventricle and cerebellum (Fig. 2).

Conclusion

This study represent first application of cryoprobe technology in conjunction with high field strength in neurobiological research using zebrafish. Improved S/N ratio and possible reduction in experimental time opens the possibility to study brain structure in living zebrafish in future.

References:

(1) Joore J, Timmermans A, et al. *Biochemistry and Cell Biology* 75, 601-612 (1997); (2) Tropepe V, Sive HL. *Genes Brain Behav.* 2, 268-81 (2003)

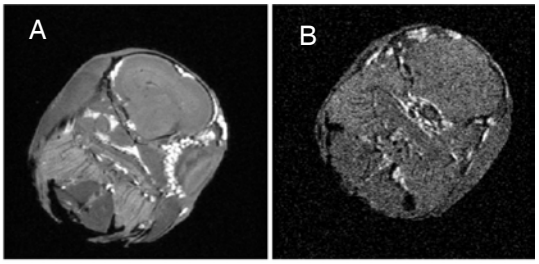

Fig. 1: Images from the head of adult zebrafish obtained by using micro-imaging cryoprobe (A) and conventional Micro2.5 probe (B) at 9.4T. Slice in coronal plane were obtained using 3D MSME pulse sequence (TE= 5.4 ms; TR= 1800 ms; ns=4; T). The image resolution is 43 μm.

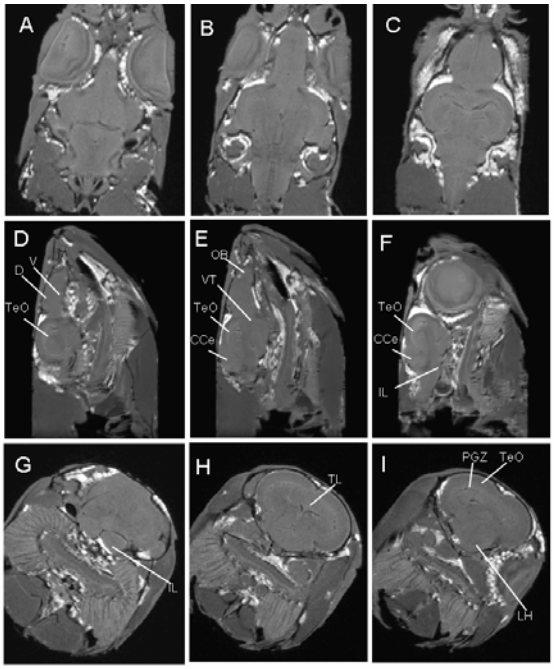

Fig. 2: Images from the head of adult zebrafish showing anatomical details in the brain obtained by using micro-imaging cryoprobe at 9.4T. Slice in axial plane (A-C), sagitall plane (D-F) and coronal plane (G-I) were obtained using 3D MSME pulse sequence (TE= 5.4 ms; TR= 1800 ms; ns=4; T; Scantime 31 min). The image resolution is 43 μm. V, ventral telencephalic area; D, dorsal telencephalic area; opticum tectum; OB, olfactory bulb; VT, ventral thalamus; CCe, cerebellar corpus; IL, inferior hypothalamus; TL, longitudinal torus; LH, lateral hypothalamic nucleus.