

Poster Only

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Prefer Traditional Poster

Three-dimensional stereotaxic atlas of the Mozambique Tilapia (*Oreochromis mossambicus*) using High-Resolution MRI.

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Introduction:

The Mozambique tilapia, *Oreochromis mossambicus*, is a highly social cichlid species that has been used as a model system in a wide range of studies, related with immunology¹, toxicology, endocrinology^{2,3}, physiology⁴, endocrinology, among others. The increasing number of genetic tools available for this species, together with the emerging interest in its use for neurobiology studies, increased the urgency in an accurate hodological mapping of the tilapia brain to complement the available histological data.

Methods:

(1) **Specimen preparation:** To collect MRI images, three adult tilapia females (standard length: 10.7±1.8cm) were perfused transcardially, first with a phosphate-buffered saline solution (PB 0.2 M), to clear the vasculature, followed by a solution of Paraformaldehyde (2 %) in Dotarem® (1 %). For scanning, the brains of the fish were removed from the skull and transferred to a polypropylene tube filled with Fluorinert®, a proton-free susceptibility-matching fluid.

(2) **Ex vivo scans:** MRI scanning was performed on a 9.4T horizontal bore Magnetic Resonance Imaging system (Bruker BioSpin MRI GmbH, Ettlingen, Germany) using the standard Bruker cross coil setup, being a quadrature transmit volume coil (inner diameter 72mm) and a quadrature receive surface coil, designed for mice brain. Horizontal images of the Tilapia brain were acquired using a fat-suppressed T₂-weighted three-dimensional RARE sequence with the following parameters: acquisition bandwidth of 33kHz, TE/TR=30/350ms, echo train length=2, 8 averages, a field of view of (13.5×8×10)mm³ and an acquisition matrix of (270×160×200), resulting in a nominal spatial resolution of (50×50×50)µm³. The total acquisition time was 12.6 h.

(3) **Manual segmentation:** Brain and nuclei delineation was done manually using Amira software (Mercury Computers Systems, USA). Segmentation was done slice-by-slice in a coronal perspective and posteriorly confirmed systematically in the two other orthogonal views (axial and sagittal). Major brain subdivisions (Telencephalon, Diencephalon, Mesencephalon, Rombencephalon) were first delineated, followed by structures which presented more distinct boundaries (e.g. olfactory bulbs, optic tectum and corpus cerebellis), which helped identifying smaller nuclei. In addition, histology slices were used as guidelines for the location and boundaries of smaller structures. In this atlas, we adopted the nomenclature for brain structures from Wullimann et al.⁵ and Meek and Nieuwenhuys⁶.

Results:

Using high resolution MRI, we acquired a detailed digital tilapia brain atlas, depicting several major and minor structures (Figure 1). Supported by an histological map of this species, which was also purposely developed for this study, we identified a total of **55 structures** at an isotropic resolution of 50µm (major structures represented in Figure 2). Using the intrinsic three-axis nature of MRI-based atlases, we established a stereotaxic coordinate system which gives the centre coordinates for each structure with respect to the reference point – i.e. intersection between the mid-sagittal and the mid-horizontal planes and the Anterior Commissure.

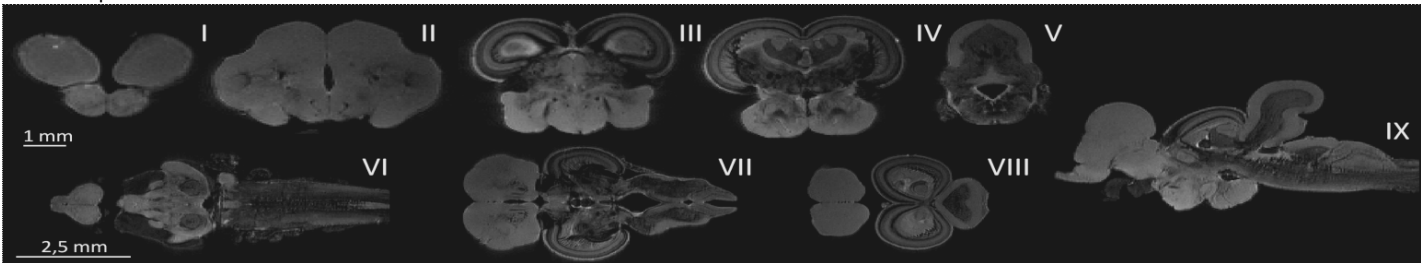


Fig. 1 – High Resolution MRI images displaying coronal (I-V), horizontal (VI-VIII) and sagittal (IX) slices of the tilapia brain. Coronal images are ordered from anterior to posterior; horizontal images are ordered from ventral to dorsal; and a mid-sagittal slice of the brain is displayed.

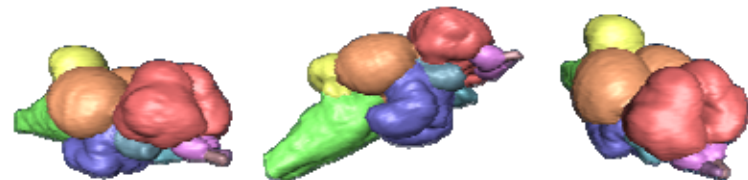


Fig. 2 – 3D surface rendered representation of the major structures of tilapia brain segmented on the MRI images using Amira software.
■ – Olfactory bulbs; ■ – Telencephalon; ■ – Diencephalon;
■ – Optic Tectum; ■ – Cerebellum; ■ – Brain Stem;
■ – Olfactory Nerves (left and right); ■ – Optic Nerves (left and right).

Conclusion:

In this study, we elaborated a three-dimensional, high-resolution digital atlas using magnetic resonance imaging. This high resolution tilapia brain atlas is expected to become a very useful tool for neuroscientists using this fish model and will certainly expand their use in future studies of the CNS. Also, the near future possibility of acquiring precise stereotaxic coordinates and the identification of relevant brain nuclei will make this an indispensable tool.

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References: [1] Wu CC et al. (2010), Fish and Shellfish Immunology 29 (2), 258–263. [2] Watanabe S. & Kaneko, T. (2010), General and Comparative Endocrinology 167 (1), 27–34. [3] Antunes RA & Oliveira RF (2009), Proceedings of the National Academy of Sciences U.S.A. 106, 15985–15989. [4] Breves JP et al. (2010), Molecular & Integrative Physiology 155 (3), 294–300. [5] Wullimann M et al. (1996), Basel: Birkhauser Verlag. [6] Meek J & Nieuwenhuys R(1998), In: Nieuwenhuys R, Ten Donkelaar HJ, Nicholson, editors. The central nervous system of vertebrates. New York: Springer-Verlag. 758–937.

Reviewer Comments for Abstract: 2031

Additional Recomendations for Abstract: 2031

Reviewer 1: No Additional Recommendations

Reviewer 2: N/A

Reviewer 3: N/A

Reviewer 4: N/A

Reviewer 5: No Additional Recommendations