

# Using MRI to quantify forebrain development of a common neurobehavioral animal model, the zebrafish (*Danio rerio*), reared in different conditions

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## Introduction:

Zebrafish are a popular model for neurobehavioral research, yet very little work has addressed how housing conditions in the laboratory and daily handling stress can affect their brain development. In fish, similar to mammals, neurogenesis in the forebrain is regulated by extrinsic environmental factors as well as intrinsic factors such as stress(1,2). Therefore, it is of interest to consider how a combination of environmental enrichment and exposure to stress might affect forebrain development in zebrafish. MRI is needed to effectively detect gross changes in forebrain volume as well as provide a three-dimensional visualization of the data.

## Subjects & Methods:

Zebrafish (25 days post fertilization) were kept in four different environmental conditions: (i) Enriched+Chased, (ii) Enriched, (iii) Plain+Chased, and (iv) Plain. The enriched tanks contained plastic plants, a refuge (black plant pot), a novel object (chosen for their responsiveness not for their naturalness; elbow pipe, pink rock or a plastic bottle) a biofilter, heater and gravel substrate. The plain tanks contained a heater and biofilter only. Enrichment was moved around once a week and rotated every week to give more variation in the environment. The Enriched+Chased and Plain+Chased treatments only were subject to chasing with a small dip net every day. After 78 days the fish were euthanized (buffered 2g/L MS-222), rinsed and fixed (5 ml 10% neutral buffered formalin) for 72 hours and immersed in phosphor buffered saline containing 2% Magnevist for 7 days. The high concentration of Magnevist allowed for a short repetition time and a high contrast in the imaging. A standard 3D spin echo sequence with an isotropic resolution of 20  $\mu$ m comprised a field of view of 10 x 4 x 3 mm and a matrix size of 500 x 200 (0.75 partial Fourier = 150) x 150. With 32 averages and a repetition time of 70 ms (echo time 8.85 ms) the total scan time was 14 hours. During

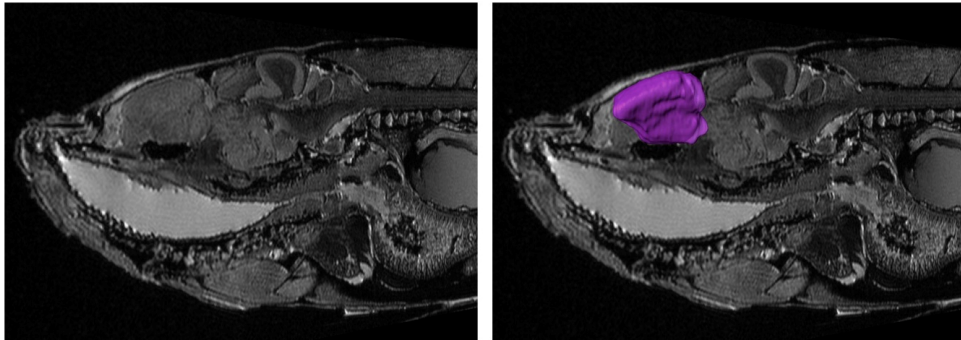


Fig. 1 (a) a representative image of the zebrafish from the three dimensional MRI data set and (b) the same image with a surface view of the whole telencephalon segmentation.

reconstruction (Matlab; The Mathworks, Inc., Natick, MA) zero-filling by a factor of two in each direction resulted in a pixel resolution of 10  $\mu$ m isotropic. Image segmentation of the telencephalon (involved in spatial learning and modulating the stress response) was performed using Avizo (VSG3D, USA). Segmentations were verified using a variety of neuroanatomical references (3,4). Each fish was segmented three times and an average volume was calculated.

## Results:

MRI provided detailed information on zebrafish neuroanatomy in three-dimensions, and allowed accurate quantification of telencephalon volume. (Fig. 1a,b). Due to the high contrast and resolution the three segmentations performed on each brain had only small variations (< 3%). Telencephalon volume relative to whole fish standard length was significantly different across treatments (Fig. 2;  $F_{3, 20} = 5.40$ ,  $P < 0.01$ ). Enriched fish had the largest telencephalon volume compared to all other treatments.

## Discussion:

MRI provides an excellent tool to detect anatomical changes in the development of the zebrafish brain as a result of environmental influences. The MRI data showed that enrichment alone led to an increase in telencephalon volume. Enrichment had a positive effect on neural growth, but this effect was decreased when stress was added. The data further support behavioral differences that found a significant role played by enrichment (5). Better knowledge of the effect of housing conditions on forebrain development in laboratory fish is needed to have a better understanding of the impact of different husbandry procedures. Given the role of zebrafish in biomedical studies, knowing how their rearing environment shapes brain development is important. Future studies should explore whether other key brain structures (e.g. cerebellum, thalamus etc.) are affected by rearing environmental conditions at different stages of development.

## References:

[1] von Krogh *et al.* (2010). *Physiol Behav*, 101, 32-39. [2] Johansen *et al.* (2012). *Comp Biochem Phys D*, 7, 161-171. [3] Ullmann *et al.* (2010). *Neuroimage*, 51:76-82. [4] Wulliman *et al.* (1995). 'Neuroanatomy of the Zebrafish Brain: A Topological Atlas', Birkhaeuser. [5] Grassie *et al.* (in review). *Anim Behav*.

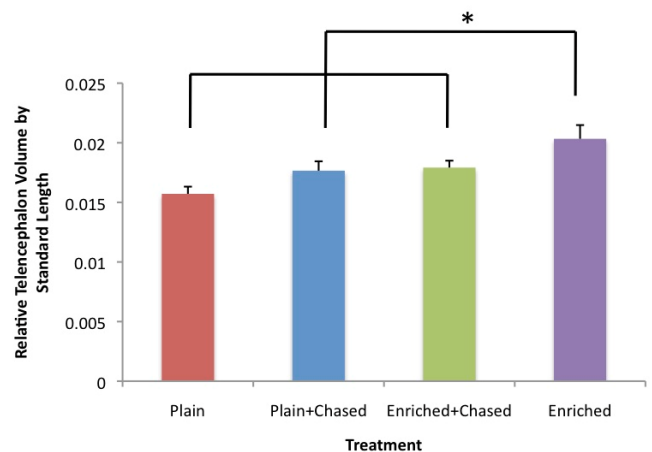


Fig. 2: Relative telencephalon volume for each treatment (mean  $\pm$  S.E.M.). \* Indicates significant difference.