Functional Brain Imaging and Targeted Lesion Studies Using Manganese-Enhanced MRI and Focused Ultrasound in Non-Conventional Model Species

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Introduction: Investigating the neural mechanisms underlying behavior in non-model organisms remains a major challenge due to the lack of suitable neuroimaging and molecular tools. Traditional techniques such as electrophysiological recordings and immediate early gene (IEG) mapping depend heavily on established stereotaxic brain atlases or species-specific antibodies, which are generally unavailable outside classical model organisms like rodents or zebrafish.

Surgical lesion studies, often used to confirm causal relationships between brain regions and behavior, face significant obstacles in species lacking stereotaxic coordinates or in aquatic animals where surgical environments are difficult to control. As a result, the scope of comparative neurobiology is often limited to a narrow group of tractable species.

To overcome these limitations, this study presents a protocol combining Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) [1] for functional imaging with Magnetic Resonance-guided Focused Ultrasound (MRgFUS) for targeted, non-invasive brain lesions. This integrated approach is demonstrated in *Amatitlania nigrofasciata* (convict cichlid), a freshwater fish, and is adaptable to a wide range of species, offering new avenues for neuroethological and comparative research.

Methods:

MEMRI Functional Imaging: $MnCl_2$ is a contrast agent taken up by active neurons. We first optimized the $MnCl_2$ dosage and administration route by testing intraperitoneal and intramuscular injections at 50 or 75 mg/kg $MnCl_2$. Based on these results, our final experimental protocol employed intraperitoneal injections of $MnCl_2$ at a dose of 50 mg/kg. Post-injection, fish participated in repeated behavioral sessions involving a problem-solving task to promote behavior-linked manganese accumulation. Forty-eight hours later, animals were anesthetized and imaged on a 17.2 T Bruker Biospin MRI scanner using a volume transmit/receive coil with inner diameter of 45 mm. For manganese accumulation quantification we acquired T1 weighted images at 80 µm in plane resolution. Other relevant acquisition parameters are as follows: TR/TE 250/2.8 ms, 24 slices, slice thickness 0.2 mm, 40 averages. T1-weighted images were analyzed using retina-based normalization to compare manganese accumulation across brain regions.

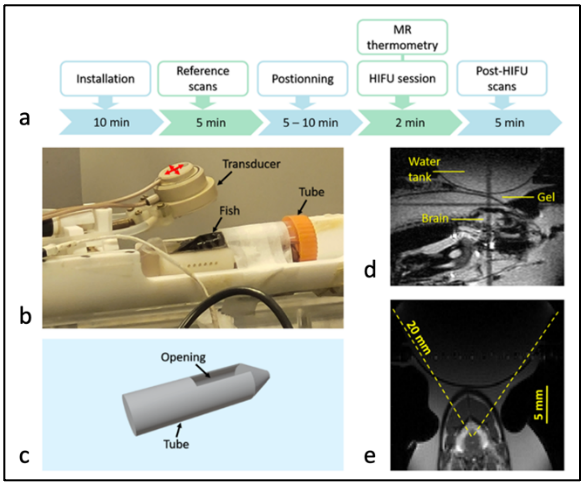

Figure 1. Experimental setup for simultaneous HIFU and MR imaging. a. Workflow of the study. b. MR and ultrasound setup. The transducer can move along the two perpendicular directions symbolized by the red arrows. c. The fish was placed in the tube and maintained in water under anesthesia. An opening was created to apply transducer on the fish head. Sagittal T1-weigthed (d.) and coronal T2-weigthed (e.) images.

HIFU Lesion Protocol: To validate functional imaging results, MRgFUS was used to induce thermal lesions in targeted brain areas [2]. An MR-compatible ultrasound transducer, placed in a 7T MR scanner [3], delivered 15-second sonications to the cerebellum with an estimated acoustic pressure of 6 MPa. The focal spot was positioned using T2-weighted MR images (Figure 1). Real-time MR thermometry monitored tissue heating (~18°C increase), and T2-weighted scans taken 48 hours post-sonication assessed lesion formation. Visual confirmation was obtained via post-mortem inspection of formalin-fixed brains.

Results and discussion:

Intraperitoneal injection proved more effective than intramuscular administration, and the 50 mg/kg dose offered a reliable balance between contrast and animal safety (Figure 2). MEMRI enabled non-invasive visualization of behaviorally induced brain activity at sub-millimeter resolution. Manganese uptake was robust in regions associated with task engagement, with significantly higher signal intensities in the inferior lobe (mean relative intensity: 7139±388, n=10) of trained fish compared to controls (mean relative intensity: 6422±425, n=10, p<0.001).

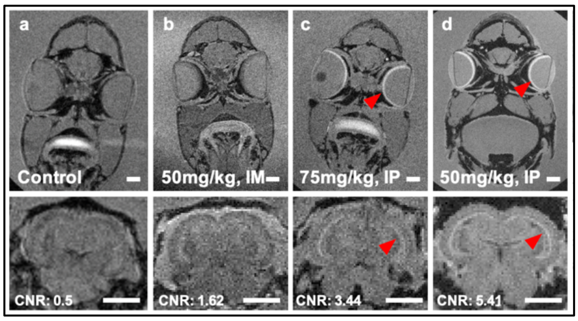

Figure 2. Manganese dosage for MEMRI in convict cichlids. T1 weighted images. a-d: Intraperitoneal injections provide better signal than intramuscular injections for similar manganese concentrations. Scale bar: 2.5mm.

HIFU induced well-localized brain lesions in the cerebellum without compromising overall health or motor abilities, allowing continued participation in behavioral tasks. MR thermometry effectively tracked focal temperature increases, while T2 signal changes and macroscopic hemorrhage confirmed lesion formation (Figure 3). Lesions in the inferior lobe, a region activated during task performance, resulted in impaired behavioral responses, validating its role in the task and demonstrating the utility of HIFU for causal studies of brain function. This dual approach addresses several limitations of traditional methods: it eliminates the need for surgical procedures or transgenic lines, allows precise targeting of deep brain structures, and can be implemented in species lacking detailed neuroanatomical data.

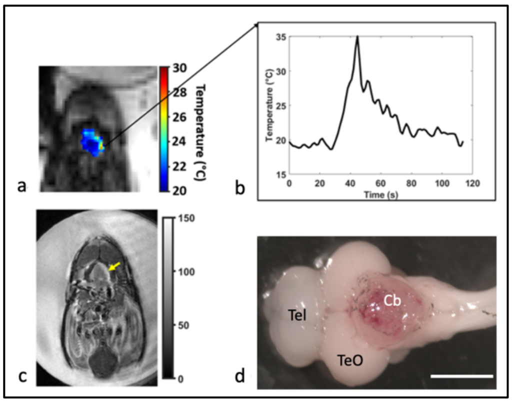

Figure 3. Thermal ablation of the cerebellum. a. Thermometry map of the cerebellum where a temperature rise was detected on the left. b. Temperature evolution over time during MR thermometry scan time. c. An hyperintense T2w signal was observed, 48 hours after HIFU protocol, at the location of the temperature rise indicated by the yellow arrow. d. Dorsal view of a lesioned fish brain, showing extensive hemorrhage in the corpus cerebelli (Cb).

Conclusion: The combination of MEMRI and MR-guided HIFU represents a transformative toolkit for neuroethological research in non-conventional species. MEMRI enables functional mapping of neural activity linked to natural behaviors, while HIFU allows precise, non-invasive lesions to test causal involvement of specific regions. This methodology can be broadly applied to species across the vertebrate and invertebrate spectrum, including those lacking established genetic or anatomical resources [3]. By facilitating functional brain imaging and lesion studies in previously inaccessible species, this approach opens new avenues in comparative neuroscience and evolutionary biology.

References: [1] Radecki et al., PNAS (2014). [2] Dervishi et al., Int. J. Hyperthermia. (2013). [3] Magnin et al., J Ther Ultrasound (2025). [4] Wagner, Egelhaaf & Carr, J. Comp. Physiol. (2024).