PhD Thesis Title: Brain Magnetic Resonance Imaging for Investigation Hearing Loss and Environmental Enrichment

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ABSTRACT:

Magnetic resonance imaging (MRI) is an established radiology method that provides excellent soft-tissue contrast without requiring ionizing radiation. As a result, MRI has found many applications in clinical and basic research fields, such as neurology and neuroscience. However, MRI images have traditionally been assessed qualitatively by experts. In recent years, extensive effort has been made to develop quantitative image analysis methods to improve reproducibility and facilitate the cross-studies assessment. During my PhD studies, I developed brain magnetic resonance imaging (MRI) analysis methods. In this thesis, I will present my two main studies involving these methods. One was conducted in humans and the other in animals. The imaging methods were combined with other research methods where suitable, resulting in rich multi-modality studies.

The first study was a meta-analysis of all published MRI studies about hearing loss. Hearing loss is a heterogeneous disorder thought to affect the brain's reorganization across the lifespan. The exact structural endophenotype of hearing loss is not known, although it is assumed to affect the auditory regions of the temporal lobe, such as Heschl's gyrus. We assessed the structural alterations of hearing loss by using a meta-analysis of effect size measures based on MNI coordinate mapping of MRI studies. The unique effect size metrics based on Cohen's d and Hedges' g were created to map the coordinates of gray matter (GM) and white matter (WM) alterations from bilateral congenital and acquired hearing loss populations. Three mapping techniques were used and compared: coordinate-based anatomic likelihood estimation (ALE), multi-level kernel density analysis (mKDA), and seed-based d Mapping (SDM). Using a meta-regression, GM and WM trajectories were mapped across the lifespan to visualize the progression of hearing loss throughout the lifespan. Heterogeneity in effect size metrics was determined using the forest, Baujat, Funnel, Galbraith, and bubble plots to discern dispersion and spread of data points. Lastly, I displayed an endophenotype map of hearing loss alterations in GM and WM obtained from a multivariable meta-regression of the effect size. The systematic review and meta-analysis identified n = 72 studies with structural alterations measured with MRI (bilateral=64, unilateral=8). The bilateral studies contained more than 28000 variable datapoint metrics broadly categorizing hearing loss into congenital and acquired cases (n = 7445), and control cases (n = 2924), with ages of 34.92 ± 23.08 and 30.61 ± 19.45 years, respectively. We found that hearing loss affects GM and underlying WM in nearly every region of the brain. In congenital hearing loss, GM decreased mostly in the frontal lobe. Acquired hearing loss similarly had a decrease in the frontal lobe GM, albeit the insula was most decreased. Congenital white matter underlying the frontal lobe GM mostly decreased. The temporal lobe had different GM alterations in congenital and acquired depending on the region. The WM alterations most frequently underlined GM alterations in congenital hearing loss, while the acquired hearing loss studies did not assess the WM metric frequently. There were also several limitations of the studies in the hearing loss field with manuscripts. For example, not reporting the mean and standard deviation SD for GM or WM metrics, lack of MNI coordinates, and some not reporting sufficient control populations. These factors could have contributed to heterogeneity as the underlying explanatory

variables. The present article presents a novel 'hit-enter' repeatability format for assessing the hearing loss, providing all data, scripts, and analysis from data curation to visualization available for reproducibility. Future studies should use the endophenotype of hearing loss as a prognostic template for discerning the impact and clinical outcomes.

The second study explored the circadian dependence of environmental enrichment. Environmental enrichment induces functional-neuronal changes, but the initiation of the cascade is unknown. We ascertained the critical period of divergence between environmental enriched (EE) and standard environment (SE) mice using continuous infrared (IR) videography, functional connectome magnetic resonance imaging (fMRI), and neuron level calcium imaging. Naïve adult male mice (n = 285, C57BL/B6, postnatal day 60) were divided into SE and EE groups. We assessed the motion activity using a novel structural-break test which identified changes in circadian and day-by-day motion activity. fMRI mapped brain-wide responses using functional connectome analyses. Awake calcium imaging was performed on the dorsal CA1 pyramidal layer based on connectome findings. We found the preeminent behavioral feature in EE was a forward shift in the circadian rhythm, elongation of activity in the dark photoperiod, and overall decreased motion activity. The crepuscular period of dusk was the critical period of divergence between EE and SE mice. The functional processes at dusk in EE included increased seed functional connectivity in the visual cortex, motor cortex, retrosplenial granular cortex, and cingulate cortex. Network statistics found an increased functional connectome in EE in two hubs: the hippocampal formation and isocortical network. These hubs experienced a higher node degree and significantly enhanced edge connectivity. Follow-up calcium imaging revealed increased spike rate and maximum firing rate in the dorsal CA1 pyramidal layer, in addition to anterior-posterior and medial-lateral effect size differences between EE and SE. The emergence of functional-neuronal changes in the hippocampus and isocortex is key to the circadian shift during dusk. This finding opens many research pathways to better understand the functional-neuronal basis of environmental enrichment.

The above two studies illustrated the power of advanced image analysis methods as a complement to established research methods. In particular, as imaging is able to simultaneously assess the entire brain, we were able to identify brain regions associated with a neurological disorder that was not part of conventional understanding. This paves the way for cell and molecular-specific methods such as calcium imaging to further probe the newly identified regions. Imaging also opens new research avenues for ourselves and others to explore.

References to author publications that relate specifically to the dissertation:

1. **Manno FAM**, Rodríguez-Cruces R, Kumar R, Ratnanather JT, and Lau C, "Hearing loss impacts gray and white matter across the lifespan: Systematic review, meta-analysis and meta-regression." NeuroImage. 2021 May 1;231:117826. doi: 10.1016/j.neuroimage.2021.117826. Epub 2021 Feb 4. PMID: 33549753; PMCID: PMC8236095.
2. **Manno FAM**, Manno SHC, Ma V, Barrios FA, Cho WC, Cheng SH, and Lau C, "Simple Surgical Induction of Conductive Hearing Loss with Verification Using Otoscope Visualization and Behavioral Clap Startle Response in Rat." J Vis Exp., 2019 Oct 26;(152). doi: 10.3791/57993. PMID: 31710027.
3. **Manno FAM**, An Z, Kumar R, Wu EX, He J, Feng Y and Lau C, "Structural Alterations in a Rat Model of Short-Term Conductive Hearing Loss Are Associated With Reduced Resting State Functional Connectivity." Front. Syst. Neurosci., 2021 August 12;15:655172. doi: 10.3389/fnsys.2021.655172