Design of stereotaxic head holders for rodent fMRI

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INTRODUCTION

With its inception, functional MRI (fMRI) has enabled neuroscientists to probe the neuronal activity of brain regions *in vivo* and non-invasively. fMRI relies on the blood-oxygen-level dependent (BOLD) contrast signal to determine the areas of brain activity and therefore conclude which portions are affected by neurological or neuropsychiatric disorders. Another powerful capability of fMRI lies in its translational value, allowing researchers to study humans and animals with the same technology.

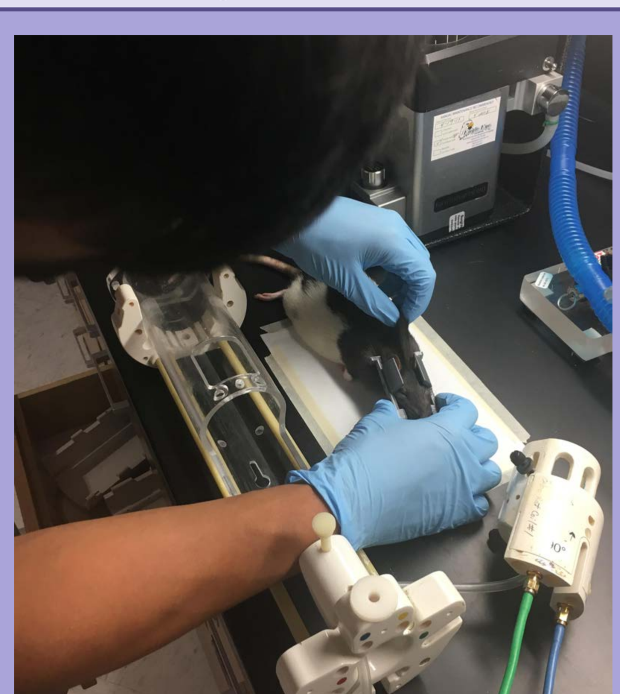
In animal studies a large problem lies in our ability to communicate the importance of the reduction of subject motion during the experiment. Animal movement introduces spin-history and motion artifacts, making it difficult to standardize the data over the entire fMRI experiment. Clearly, the issue of reproducibility arises from this complication; movement is variably dependent on animal temperament and size, probe size, etc. To mitigate these complications intuitively necessitates animal training and the creation of a headpiece that restricts movement of the head such that the brain of the animal is not only within the general center of the imaging device, but also in the center of the probe.

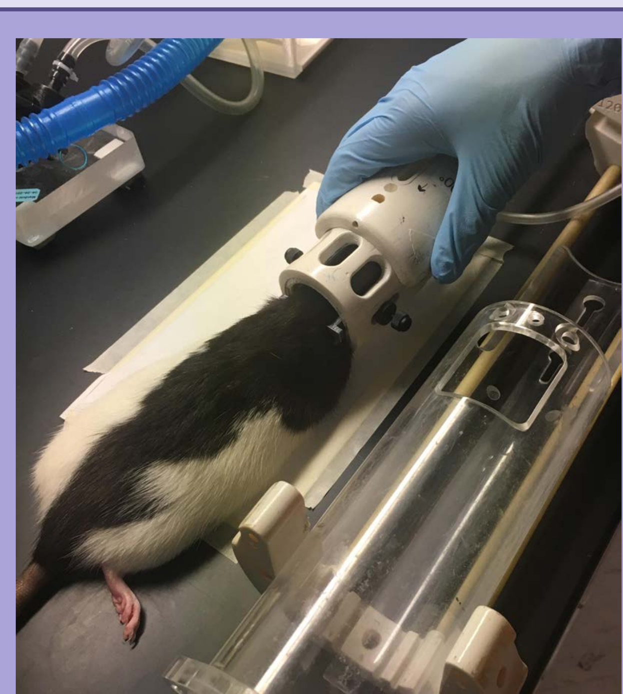
OBJECTIVES

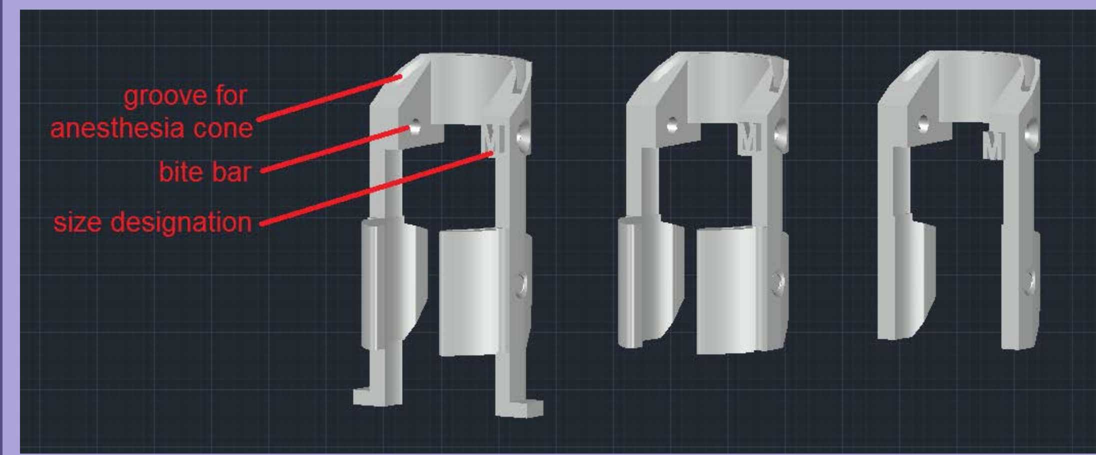
- Design and polish multiple practical head holders for rodent fMRI for various scanning protocols using 3D modelling and printing
- Learn to operate the Agilent 4.7T MRI Scanner using VnmrJ software
- Train rodents for awake fMRI and perform an awake fMRI experiment
- Perform MRI experiments looking at auditory stimulation in resting state rodents using the proper head holder

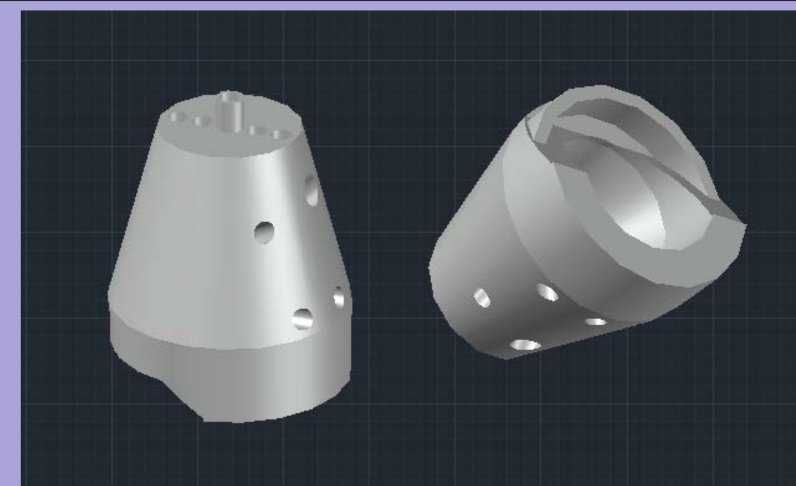
METHODS

All scans were performed using the Agilent 4.7T using VnmrJ software. The headpieces were all designed in AutoCAD 2017, tested for errors using Netfabb and Print Studio, and printed using a MakerGear M2 3D Printer with the Simplify 3D software. Auditory stimulation studies were performed by inserting thin tubes into the ear canal of anesthetized rodents and looped around to an audio frequency generator at 56 kHz which was played after a 4 minute off period and then at a 30 second on, 1 minute off scheme for the remainder of the scan. Awake fMRI required the training of the rodents to acclimate them to the MRI environment in order to reduce movement. This involved the placement of anesthetized rodents into the probe and into a dark environment. They were taken off anesthesia and allowed to wake up while recorded audio of the MRI scanner was being played. This training went on for four days before the final scan, at durations of 30, 45, 45, and 50 minutes. Similar protocol was used in the final scan. The rodents were anesthetized using isoflurane and placed into the probe and scanner and weaned off the anesthesia for scanning. All rodents used were Charles River Long-Evans rats.



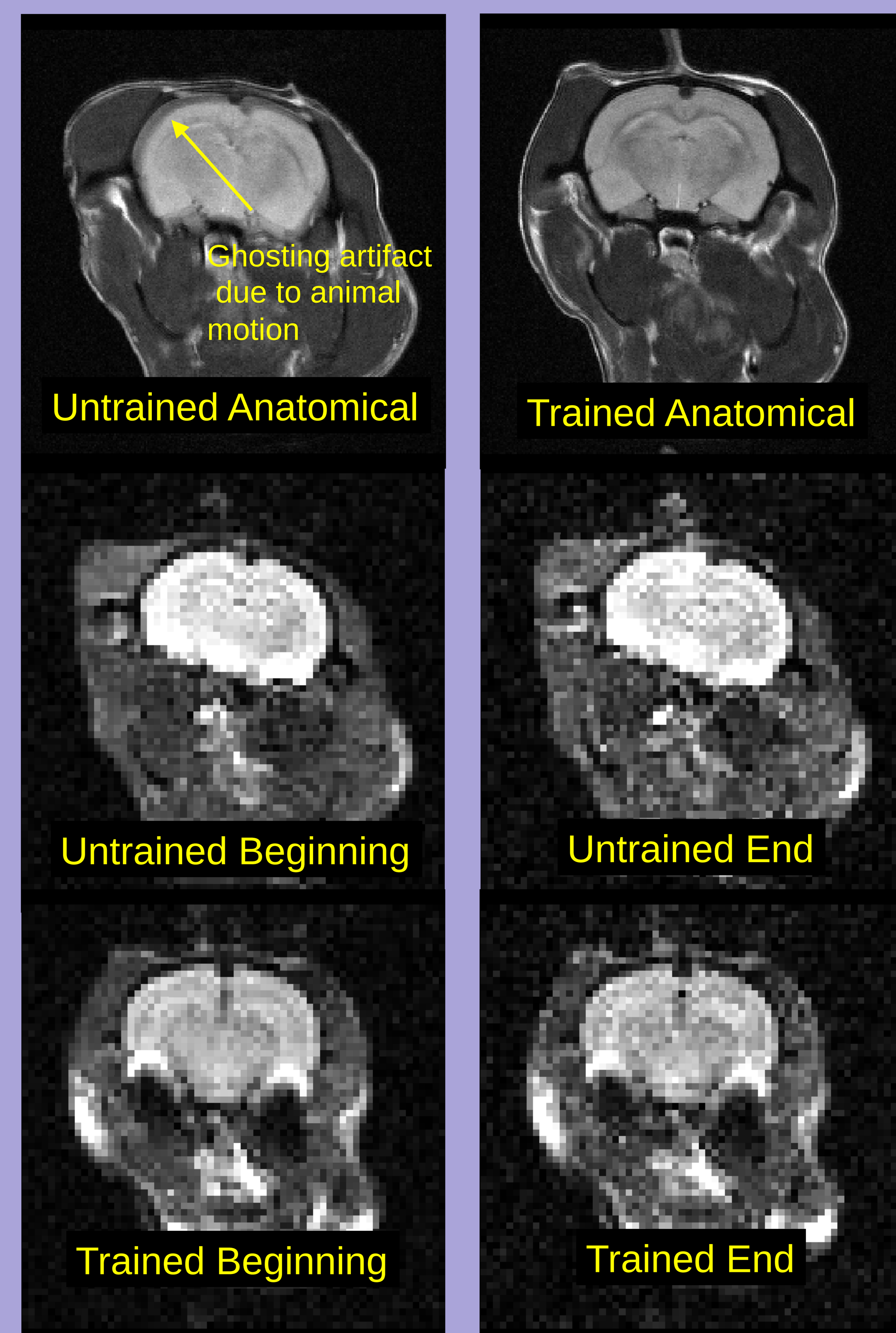






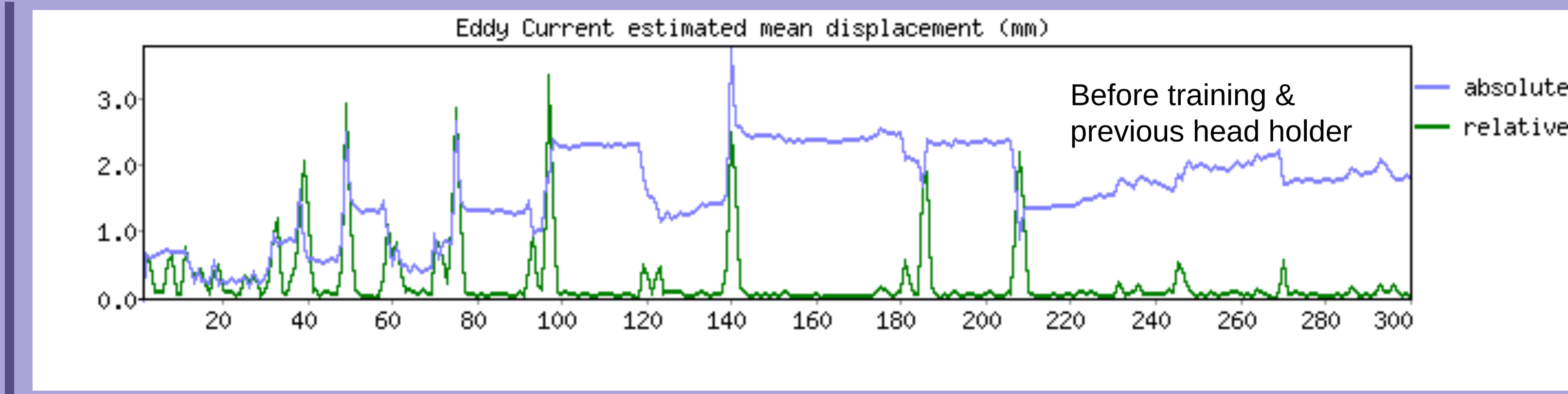
AWAKE fMRI

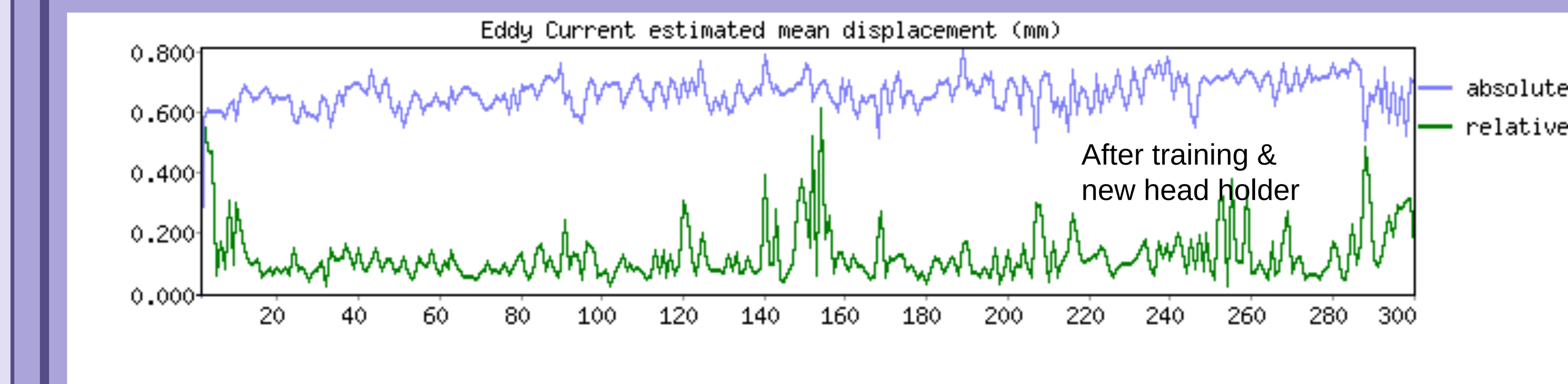
Picture right are the scans comparing movement before and after training/using the evoked head fMRI head holder. The top two scans show a beginning and end slice of the untrained rats using the previous head holder. There is visible translation of the brain, indicating movement. The middle two scans show a beginning and end slice of trained rats using the designed head holder. There is almost no noticeable change in the brain's orientation. The bottom pair of images show a slice of the preliminary anatomical scan for the pre-training/head holder and post-training/head holder. The before image shows much more ghosting and noise than the after image, which is much more crisp. The two graphs at the top of the third column show mean displacement of the scanned slices after movement correction using FSL Eddy Correct, with the upper graph showing the pre-training/head holder scan and the lower graph showing the post-training/head holder scan. Whereas the upper shows large spikes (~3mm) that can be attributed to the rodent pushing and twisting in the probe, the lower is much more stable, with just small fluctuations (~0.8mm).



MODELS

Shown left are the three designed headpieces and view from above and below the anesthesia cone. The wedge of the anesthesia cone allows for a modular fit into the groove of the body piece and has a protruding opening that allows for the fitting of an anesthesia tube. From left to right, the body pieces are for awake fMRI, resting state fMRI, and evoked fMRI. The awake fMRI requires the most restraints to contain movement, requiring the use of the end legs. The resting state fMRI does not need these legs due to the use of anesthesia. The piece for evoked fMRI requires the removal of the upper restraint, because there needs to be open access to various appendages (e.g. ears in auditory fMRI). The various holes in the anesthesia cone exist for the same reason. The third figure depicts the various sizes, with all measurements being in millimeters. Because data reliability is strongly dependent on movement, the better the headpieces fit, the less artifacting occurs and the more useful and accurate the data.





AUDITORY

Unlike Wu et al. which found an increase in BOLD signal in the contralateral inferior colliculus and lateral lemniscus at 22 kHz, we did not see similar results at 56 kHz after NODDI analysis. We believe this to be because our long, narrow, and flexible tubing resulted in little to no audio reaching the rat. While the head holder functioned as intended, improvements need to be made in regards to the audio aspect.

CONCLUSION

During this summer, I created and tested many head holder variations to account for animal size and imaging technique. The headpieces were designed for several fMRI studies: resting state fMRI (e.g. anesthetized rodents), awake fMRI, and evoked fMRI (e.g. normal auditory and visual stimulation, and facial pain stimulation). These modular headpiece variations were created in Autodesk AutoCAD 2017 and printed using a MakerGear 3D printer. These various headpieces in conjunction with training enabled an increase of the scan precision and aided in efficiency and reproducibility in both awake and anesthetized animals.

REFERENCES

1. Wu, E. X., Gao, P. P., Zhang, J. W., et al. (2015). Auditory midbrain processing is differentially modulated by auditory and visual cortices: An auditory fMRI study. *NeuroImage, 123*, 22-32. doi:10.1016/j.neuroimage.2015.08.040

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