Brain Detox: Investigating Ultrasound-Enhanced Glymphatic Waste Removal

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Our brains have a built-in cleaning system that helps clear out waste. But in brain diseases like Alzheimer's, this system doesn't work well. I wanted to test if sound waves, could make the brain's cleaning process faster. To test this, I coded a computer program to analyze MRI scans of rat brains to demonstrate how quickly the brain removed waste with and without sound wave stimulation. I found that sound waves significantly helped the brain clean itself faster, leading to potential ways to prevent or treat brain diseases by improving how the brain gets rid of waste.

Why?

Alzheimer's is a disease that gradually erases a person's memories, making everyday life more difficult. It doesn't just affect the individual—it deeply impacts their loved ones. I wanted to explore ways to slow its progression and help people like my grandmother.

Purpose

In this study, LIFU treatment was applied to the meningeal lymphatic vessels, which serve as pathways for the outflow of waste products. I hypothesized that LIFU will mechanically enhance the speed and efficiency of waste removal in the brain parenchyma, improving glymphatic function.

Currently, only lifestyle changes like exercise have been shown to slow cognitive decline, but no direct method exists to remove toxic proteins such as amyloid beta or

tau. LIFU brain sweeping offers a new approach - actively clearing these substances and preventing the progress from early cognitive impairment to more severe conditions like mild cognitive impairment (MCI) or Alzheimer's disease (AD). This technique could be especially beneficial for older adults and individuals already experiencing early cognitive decline, offering a safer alternative to existing drug treatments, which often come with severe side effects.

How?

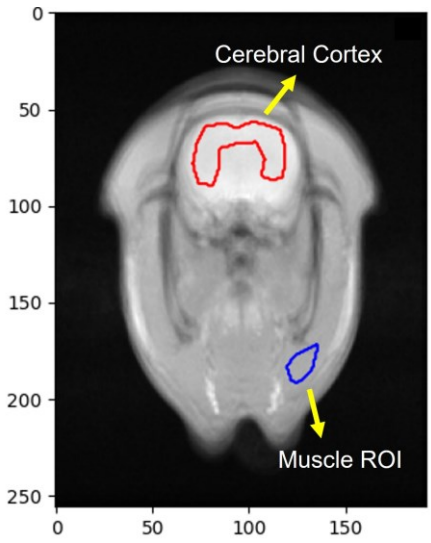
For my project, I analyzed pre-existing MRI datasets. MRI machines use strong magnets to line up tiny particles in the brain that send out signals. These signals create detailed brain images to help track how well the brain's cleaning system works. To observe clearance, a contrast agent called gadolinium was injected into the rat, going into the cerebrospinal fluid (CSF), the liquid surrounding the brain. This contrast agent acted as a marker, showing glymphatic activity in the scans for comparisons.

Dataset

The dataset consisted of MRI scans from 12 different rats, tracked across 26 time points, including a pre-experiment baseline, a scan taken thirty minutes after the experiment began, and additional scans recorded every hour for 24 hours. The data was divided into two groups: a control group that did not receive LIFU stimulation and a sweeping group that did. Both groups were subjected to nearly identical experimental conditions, including positioning, anesthesia, and MRI scanning protocol, ensuring comparability between the control and LIFU-treated rats.

Design Process

To compare the two groups, I developed a Python-based MRI data processing pipeline. The pipeline allowed me to select a brain slice where the cerebral cortex was visible. Once the optimal slice was chosen, I manually selected two regions of interest (ROIs): one in the brain, covering the hippocampus, cortex, and cerebellum, and another in the muscle, which served as a reference for background noise or systemic variations. Since the muscle is anatomically close to the brain but functionally separate from the glymphatic activity, it provided a stable comparison point.



Once the ROIs were set, the pipeline extracted key metrics to evaluate the difference in waste clearance rate among the control and LIFU datasets. It generated graphs displaying mean intensity and signal difference ratios across all time points. It also recorded the time-to-peak and peak-to-baseline hours. Once the analysis was completed, the pipeline automatically saved all calculated data into a CSV file.

What?

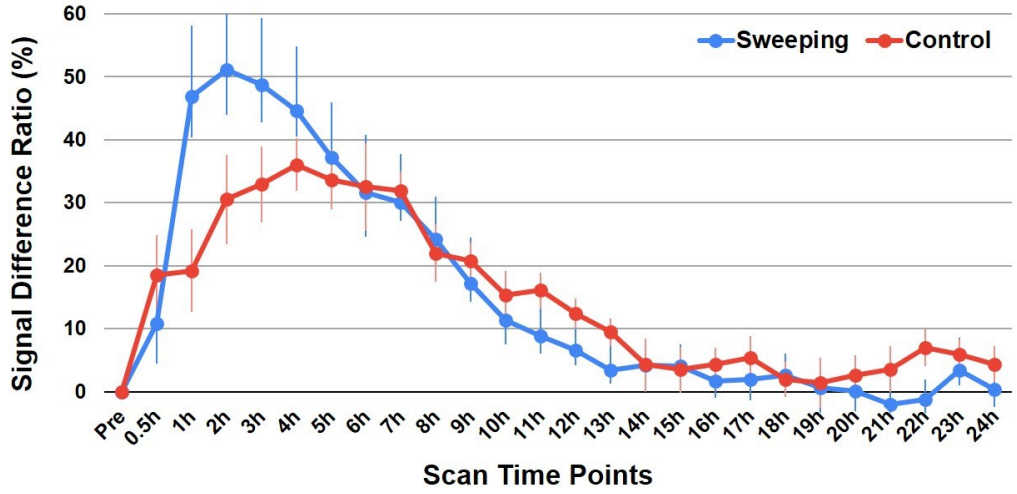
To analyze the results across 12 datasets, I programmed the code to use the Wilcoxon Ranksum Test, a **nonparametric** statistical test. I chose this method because it does not assume a normal distribution, making it suitable for small sample sizes, which consisted of six control and six sweeping subjects. Since the control and sweeping groups were composed of different individual rats, an **unpaired** test was necessary to compare the differences between them accurately. The Wilcoxon Ranksum Test determines whether one group has consistently higher or lower values than the other. For a result to be considered statistically significant, there must be a 5% or lower chance that the observed differences occurred by random chance under the null hypothesis. If the p-value is less than or equal to 0.05, the result suggests a meaningful difference between the groups.

Results

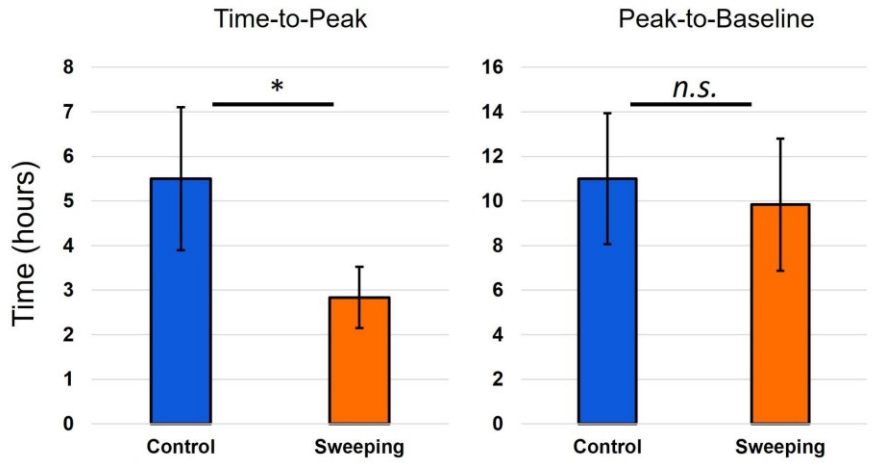
The signal ratio difference shows the relative intensity changes over time. Statistical tests computed **marginally** significant differences at 1 hour, which corresponds to the glymphatic inflow phase, and at 11, 12, 13, and 17 hours, which are associated with the

outflow phase. At the 1-hour mark, the contrast agent entered the brain more rapidly in the LIFU group (p = 0.054).

The graph visualizes the average signal difference ratio over time for both the LIFU-treated (Sweeping) and Control groups. The LIFU group had a significantly steeper rise in the first hour, reaching a higher peak intensity than the control group, indicating



enhanced glymphatic inflow (p = 0.054 at 1h).



The time-to-peak, which measures how long it took for the contrast agent to reach its highest signal intensity, significantly differed between groups (p = 0.016), further

supporting the idea that LIFU accelerated the entry of waste-clearing fluid. However, the peak-to-baseline, which tracks how long it took for the signal to return to its baseline level, did not show a strong difference between groups ($p = 0.47$). This suggests that while LIFU improved inflow speed, its impact on the removal of waste from the brain (outflow) was less pronounced. The time points 11 ($p=0.025$), 12 ($p=0.025$), 13 ($p=0.016$), and 17 ($p=0.054$) hours still showed a noticeably faster removal of the contrast agent compared to the control group. My prototype automates the entire workflow, including data import, preprocessing, analysis, and CSV output, allowing users to efficiently obtain clear, actionable results with minimal manual effort.

Conclusion

The results of this study suggest that LIFU enhances the influx phase of the glymphatic system, meaning that it may help initiate the brain's natural waste clearance process efficiently. Since prior research indicates a strong connection between glymphatic influx and efflux, an improvement in the early-phase waste uptake likely contributes to an overall increase in waste clearance. While there was no statistically significant difference in the peak-to-baseline time (efflux phase), this does not necessarily mean LIFU has no impact on waste removal. Efflux is influenced by more complex factors, and it is possible that longer duration of LIFU exposure would lead to enhanced outflow over time. Additionally, results indicate that there were notable differences during the outflow period (11-13 hours post-injection), suggesting that LIFU still plays a role in later clearance stages.

To further support researchers in this field, I plan to make my MRI data processing pipeline publicly available as a software package on GitHub, allowing others to use and build upon this tool for future studies. Ultimately, these results demonstrate that modulating glymphatic flow through LIFU stimulation has the potential to aid in the removal of toxic proteins from the brain, a critical step in developing new treatments for patients with Alzheimer's and other neurodegenerative diseases.

Future improvements

In the future, I plan to refine my MRI processing pipeline to improve automation and accuracy in selecting brain regions of interest. One major challenge was that over the 24-hour imaging period, the brain regions shifted slightly in some subjects, making manual ROI selection difficult and affecting signal intensity measurements. To address this, I aim to incorporate automated ROI selection using image processing techniques, reducing variability and improving consistency.