40 Hz light flickering as a possible treatment for Alzheimer's disease

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INTRODUCTION

Alzheimer's disease is a chronic neurodegenerative condition that impairs cognitive function and causes memory loss. Almost six million Americans suffer from Alzheimer's disease. There is no known cure. Key features of Alzheimer's disease (AD) are the accumulation of a protein called Amyloid-beta (Aβ) which eventually leads to large extracellular deposits called plaques. A second key feature of AD is the accumulation of Tau which eventually leads to large extracellular Tau tangles. Previous research found that induction of 40 Hz brain rhythms with various 40 Hz stimulation protocols leads to clearance of amyloid and tau (Iacrano, 2016) and improved cognition in mice modeling Aβ and Tau accumulation features of AD (Martorell, 2019) and more recently promising results have also been reported in human clinical trials (Chan, 2021). However, little is known about the details of such stimulation protocols, such as which features of 40Hz stimulation parameters are critical for inducing clearance of amyloid and tau, and which brain regions benefit from various stimulation protocols (and which may not). Therefore, as a first step, we hypothesize that 40 Hz light flickering (visual only protocol) can reduce Tau and Aβ aggregation in the parietal cortex of 3XTG-AD mice which have an intracellular accumulation of Aβ and tau (Stimmell et al., 2018). We also set out to confirm previous reports of clearance in the hippocampus and visual cortex using a visual-only protocol, but extended to a different mouse model and extended to conformation-specific markers of amyloid accumulation, targeting Aβ conformations that are thought to be more pathological

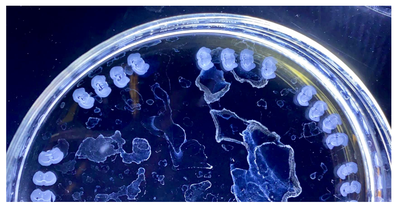

Figure 1: Brain tissue being organized for mounting and imaging after histology

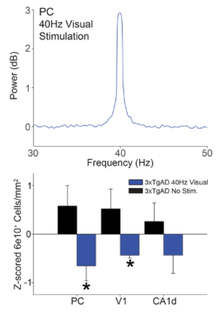

Figure 3: 6e10 results graph

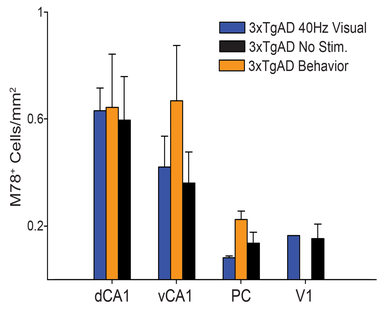

Figure 4: M78 results graph

METHODS

- In this experiment, we used 6-month-old 3XTG-AD mice. These mice are genetically modified to accumulate amyloid-beta and Tau proteins
- The mice were split into a control group and an experimental group. The experimental group received 40Hz light stimulation for 1h in an otherwise dark box once a day for seven days while control mice remained in the home cage.
- After the week of treatment, the mice were euthanized, and the brains were removed and sectioned (40μ slice thickness).
- Antibodies (M22, M78, 6e10) were used to stain and identify specific amyloid-beta segments and conformations, and antibody AT8 for a phosphorylated form of tau (pTau).
- We looked at three different regions of the brain: the parietal cortex (PC), the hippocampus (divided into ventral and dorsal subregions CA1v and CA1d), and the primary visual cortex (V1)
- Sections were stained for a neuronal marker (NeuN) visualized with a red flurophor, cell bodies of all cells visualized with a blue flurophor, and one marker of pathology (e.g., M78)visualized with a green flurophor.
- The density of neuronal cells positive for each marker of pathology will be compared between the two groups.

RESULTS

To find the results of this study we used ImageJ to manually count the neurons (labeled with the neuronal marker NeuN) that colocalized with a maker of Alzheimer's pathology. Using the ANOVA (analysis of variance) test, we examined the density of the pathology positive neurons in the experimental group (light treatment) compared to the control group (no treatment) for each brain region (parietal cortex, hippocampus, and visual cortex). This shows us how pathology varies as a function of each factor. Using a non-specific marker of Aβ ,(6e10) we found a significant reduction in 6e10 positive neuron density in the PC and V1, but not the CA1v or CA1d. Next using a conformation antibody for a more pathological conformation of Aβ , the M78 stain, we again found a reduction in Aβ density within the treatment group in all brain regions assessed, particularly in the parietal cortex; however, surprisingly, the reduction was not large enough to be statistically significant.

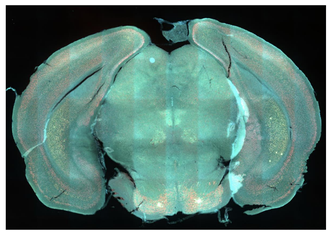

Figure 2: Scanned section with cells (blue) and neurons (red) some of which are positive for M78 (green)

DISCUSSION

This research is important because it could lead to improvements in a promising new treatment for Alzheimer's disease. Our research could help guide the human trials and future clinical trials. If light stimulation at 40Hz decreases pathology in mouse brains but only for the non-specific Aβ 1-16 6e10 staining and not for a pathological confirmation of Aβ, then this is important new information that has implications for the effectiveness of this specific treatment in humans. A direction we could take our research in the future is changing the timeframe (or other parameters) of lightbox stimulation and seeing if that alters the pattern of the results. Finally, future work can compare light only stimulation to multisensory (light and sound) stimulation to see if these different approaches are more effective at reducing M78 positive cell densities.

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