

Gamma Frequency Increase Amount of M2 Microglia and the Increase of M2 Microglia Is What Causes Reduction of Amyloid Beta

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Abstract: In the work, we try to investigate whether gamma frequency entrainment at 40 Hz can promote the transition of M1 Microglia (the brain's immune cells) towards an M2. Also, this study aims to determine if this transition enhances the clearance of amyloid-beta plaques through increased phagocytic activity of the microglia. To figure out these two experiments is designed. We create some microglia-like phenotype mice, a model for Alzheimer's disease to do the research. Finally, we get three outcomes. The first is that gamma-entrainment leads to enhanced microglial response. The gamma-entrainment group Gamma-entrainment leads to enhanced microglial response: The gamma-entrainment group showed higher expression of M2 markers (CD11b, Arg1, Mrc1), resulting in increased microglial showed higher expression of M2 markers (CD11b, Arg1, Mrc1), resulting in increased microglial activity and amyloid plaque clearance, as opposed to control and random flicker groups. Second, the gamma entrainment effect has specificity. Random flicker did not trigger significant changes in M2. Expression or plaque clearance, highlighting the specificity of gamma entrainment for amyloid expression or plaque clearance. Third, IL-4 confirms microglial role in plaque reduction. IL-4 group showed a decrease in amyloid beta. This reinforcing the idea that M2 microglia play a key role in clearing plaques, while the PBS group saw reinforcing the idea that M2 microglia play a key role in clearing plaques, while the PBS group saw no changes in amyloid levels.

Keywords: Alzheimer's disease, AD, microglia, M1, M2, gamma frequency.

1. Introduction

Alzheimer's disease (AD) is a major neurodegenerative disorder. Characterized by amyloid-beta plaque accumulation in the brain.[1] Microglia are the brain's immune cells, involved in responding to Role of Microglia. In AD, microglial activity becomes dysregulated. M2-like phenotype is anti-inflammatory, associated with tissue repair and the clearance of debris, such as amyloid-beta plaques in Alzheimer's. M1-like phenotype is pro-inflammatory, often linked to neurodegeneration and the worsening of diseases like Alzheimer's.[2] Amyloid-beta plaques are toxic protein aggregates found in Alzheimer's disease that contribute to the neurodegenerative process. And the found in Alzheimer's disease that contribute to the neurodegenerative process. And the M2-like microglia are thought to be

more efficient at clearing these plaques due to their M2-like microglia are thought to be more efficient at clearing these plaques due to their enhanced phagocytic activity.

In the work, we try to investigate whether gamma frequency entrainment at 40 Hz can promote the transition of M1 Microglia (the brain's immune cells) towards an M2. So we create some microglia-like phenotype mice, a model for Alzheimer's disease to do the research. Also, this study aims to determine if this transition enhances the clearance of amyloid-beta plaques through increased phagocytic activity of the microglia, providing potential insights into new treatment approaches for Alzheimer's providing potential insights into new treatment approaches for Alzheimer's disease.

To figure out whether gamma frequency can increase amount of M2 microglia and whether the increase of M2 microglia is what causes reduction of amyloid beta two experiments are designed.

2. Experiment 1

Investigating if gamma frequency entrainment at 40 Hz can shift microglia towards an M2-like phenotype? [3]

The objective of this experiment is to investigate whether gamma frequency entrainment at 40 Hz promotes the transition of microglia to an M2-like phenotype in 5XFAD mice[4], an Alzheimer's disease model and whether it enhances amyloid plaque clearance through increased phagocytic activity.

The mice were divided into several experimental groups which are control group, gamma-entrainment group, random flicker group and wild-type group .

The control group includes 5XFAD transgenic mice with no light stimulation. The gamma-entrainment group includes 5XFAD mice exposed to 40 Hz light flicker stimulation. The random flicker group includes 5XFAD mice exposed to random flickering patterns which is controlled for light exposure. The wild-type group serves as a blank control group.

There are some key readouts and assays we need to pay attention to.

The first one is the gene expression analysis. We can extract brain tissue from the hippocampus and visual cortex post-stimulation. By RNA-seq or qPCR we can assess M2 microglial markers (Arg1, Mrc1, CD11b) and M1 markers (TNF- α , IL-1 β , iNOS).[5] The second one is protein expression analysis. Through immunohistochemistry (IHC), we have stain for microglial markers (CD11b, Iba1), M2 markers (Arg1, Mrc1, TGF- β), and amyloid plaques (A β). Then we can evaluate microglial morphology changes linked to phagocytic activity, such as increased cell body size and reduced process length.[3] Western blot, the quantify M2 markers (e.g. CD11b, Arg1) is also worth mention. The third is the phagocytosis assay. Co-localization of Amyloid Plaques and Microglia by analyzing the extent to which microglia engulf amyloid plaques via IHC and confocal microscopy.[3] Look for increased co-localization of A β within microglia in the gamma-entrained group. We can also use flow cytometry which use FACS to measure the number of microglia engulfing amyloid plaques and express M2 markers. The last one is Amyloid Load Measurement. Use ELISA to measure soluble A β 42 and A β 40 levels in brain homogenates.[6] Measure amyloid plaque burden using anti-A β antibodies in the hippocampus and cortex.[7]

The data that need to be analyzed are as follow:

We need to analyze gene expression data, which is the outcome of the qPCR/RNA-seq. Analyze differential expression of M2 and M1 markers. Analyze the protein expression and phagocytosis assays. Quantify microglia-amyloid interactions using image analysis software. Compare amyloid load and microglial activity across control, gamma-entrained, random flicker, and pharmacological groups. Amyloid Load is also needed to be analyzed by using ELISA and IHC data to assess the effects of gamma entrainment on A β levels, and compare these results to the random flicker and control groups.

Although we did not do the experiment, we have some anticipated outcomes. In the gamma-entrainment group, higher expression of M2 markers (CD11b, Arg1, Mrc1), increased microglial engulfment of amyloid plaques, and reduced amyloid load compared to control and random flicker groups. [3,8] In the control group, low M2-like microglial marker expression and minimal plaque clearance. In the random flicker group, minimal or no significant change in M2 marker expression or amyloid clearance, indicating that the effects are specific to gamma entrainment.

3. Experiment 2

To prove the increase of m2 microglia is what causes reduction of amyloid beta.

There are three experiment processes. The experiment commenced with the meticulous segregation of laboratory mice into two distinct groups, each comprising 5-6 animals, designated as the PBS group and the IL-4[8] group. This randomization was crucial to ensure that any observed differences could be attributed solely to the experimental intervention rather than inherent variations among the subjects. Next, blood samples were meticulously extracted from each group of mice, and the concentration of A β was painstakingly measured utilizing the cutting-edge IP-MS [6] methodology. This technique, renowned for its precision and sensitivity, allowed us to accurately quantify the levels of A β present in the plasma, providing a baseline for comparison in subsequent stages of the experiment.

Second, we turned our focus to the manipulation of microglia polarization. Interleukin-4 (IL-4), a pleiotropic cytokine renowned for its ability to steer microglia towards the M2 phenotype, was meticulously dissolved in phosphate-buffered saline (PBS) to a concentration of 0.25 mg/mL and stored at -20°C to preserve its bioactivity. The choice of IL-4 was grounded in extensive literature, which consistently underscores its role in modulating the immune response by promoting anti-inflammatory and tissue-repairing functions of M2 microglia. Subsequently, using a precise microinjection pump and microsyringe, a volume of 0.5 mL of the IL-4 solution was administered to the mice in the IL-48 group. Meanwhile, the vehicle group received an equivalent volume of PBS (0.5 mL) to maintain the experimental integrity. The injection coordinates were meticulously calibrated to ensure consistent and accurate delivery of the respective solutions to the target region.

After a judiciously determined period, blood samples were once again collected from both groups of mice, and the levels of A β were reassessed using the IP-MS method. In this highly specialized assay, the detector directly measures the various species of A β , with quantitation performed against an internal standard comprising stable isotope-labeled A β . This approach not only enhances the accuracy of the measurements but also facilitates the comparison of results across different experiments.[9]

Although the experiment has yet to be conducted, we can confidently predict certain outcomes based on the existing scientific evidence. In the PBS group, where no external intervention aimed at modulating microglia polarization was introduced, we anticipate no significant changes in the quantity of A β . Conversely, in the IL-4 group, where IL-4 was administered to promote the differentiation of microglia towards the M2 phenotype, we expect a marked decrease in the quantity of A β . This reduction would provide compelling evidence in support of our hypothesis that the increase in M2 microglia is indeed responsible for the reduction of A β .

4. Conclusion

First, gamma-entrainment leads to enhanced microglial response. The gamma-entrainment group Gamma-entrainment leads to enhanced microglial response: The gamma-entrainment group showed higher expression of M2 markers (CD11b, Arg1, Mrc1), resulting in increased microglial showed higher expression of M2 markers (CD11b, Arg1, Mrc1), resulting in increased microglial activity and

amyloid plaque clearance, as opposed to control and random flicker groups. activity and amyloid plaque clearance, as opposed to control and random flicker groups. Second, the gamma entrainment effect has specificity. Random flicker did not trigger significant changes in M2. Expression or plaque clearance, highlighting the specificity of gamma entrainment for amyloid expression or plaque clearance. Third, IL-4 confirms microglial role in plaque reduction. IL-4 group showed a decrease in amyloid beta. This reinforcing the idea that M2 microglia play a key role in clearing plaques, while the PBS group saw reinforcing the idea that M2 microglia play a key role in clearing plaques, while the PBS group saw no changes in amyloid levels.

Then, how these results will impact? First, gamma-entrainment as a non-invasive therapy. The results from the gamma-entrainment group indicate that light and sound therapy (gamma-frequency stimulation) could be developed as a non-invasive hospital treatment for Alzheimer's stimulation) could be developed as a non-invasive hospital treatment for Alzheimer's disease. This could be applied in clinical settings using devices that deliver specific disease. This could be applied in clinical settings using devices that deliver specific brainwave frequencies to stimulate microglial activity and clear amyloid plaques, which offers brainwave frequencies to stimulate microglial activity and clear amyloid plaques, which offers a new therapeutic tool in hospitals and long-term care facilities. a new therapeutic tool in hospitals and long-term care facilities. second, microglia can serve as a therapeutic target. The specificity of the gamma entrainment effect, which does not occur with random flicker, suggests that targeted brainwave entrainment could selectively activate beneficial immune responses in the brain. This offers an avenue for developing treatments that precisely activate microglia to clear plaques without affecting other brain functions.

We can also see a broader impact on neurodegenerative diseases. Insights from this research may not only benefit Alzheimer's patients but could also inform treatments for other only benefit Alzheimer's patients but could also inform treatments for other neurodegenerative disorders where misfolded proteins accumulate, such as Parkinson's neurodegenerative disorders where misfolded proteins accumulate, such as Parkinson's disease and Huntington's disease.[10] disease and Huntington's disease.

Unfortunately, although we know that a 40 Hz gamma frequency will increase the amount of M2 microglia, we have not found evidence during our work to explain why the gamma frequency has to be 40 Hz.

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