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# Anti-inflammatory response controlled by gamma wave

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**Abstract:**

Since recognized as an important factor in Alzheimer's disease, microglia are often found to shift from a neuroprotective M2 phenotype to a pro-inflammatory M1 phenotype thus cause neuroinflammation. This research proposal aims to put forward a new therapy in treating Alzheimer's disease, in specific, by modulating gamma wave frequency and thereby regulate the polarization state of microglia. If it changes from M1 to M2, it changes from pro-inflammatory to anti-inflammatory, which means this strategy is efficient in treating Alzheimer's disease.

**Keywords:** Alzheimer's disease; Gamma wave; Microglia.

## 1. Introduction

Dementia is a clinical syndrome characterized by a collection of cognitive decline symptoms severe enough to interfere with daily life. Recent years, the number of individuals with dementia is keep increasing, in which Alzheimer's disease patients accounts for approximately 60% to 70% [1]. This situation undoubtedly makes Alzheimer's disease more worthy of attention. The causes and treatment methods of Alzheimer's disease have not been fully deciphered to this day. Nonetheless, with more researchers paying attention to study in Alzheimer's disease, people now recognize one of the primary features of Alzheimer's disease is Microglia-mediated neuroinflammation. In recent years, microglia—the primary immune cells of the central nervous system—have increasingly been recognized for their role in the pathogenesis of AD [2].

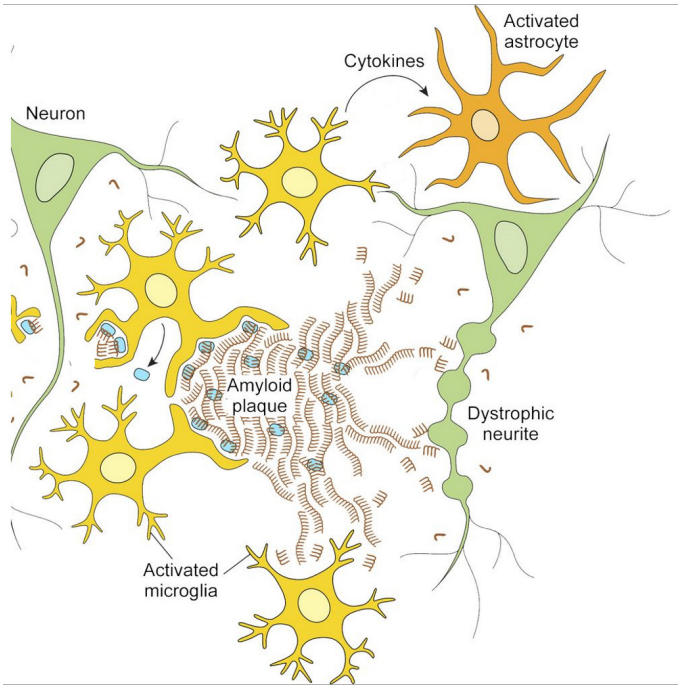


**Figure1. Depiction of microglial cellular activities related to β-amyloid pathology [2]**

Figure1 illustrates microglial cellular activities in forming deposits of Aβ into amyloid plaques, secreting factors that activate astrocytes and participating in amyloid-dependent synapse loss. These cells, which protect the healthy brain, often shift from a neuroprotective M2 phenotype to a pro-inflammatory M1 phenotype in AD patients, ex-

acerbating neuronal damage [3]. Unfortunately, previous studies have shown that M1 inhibitors are not effective in the treatment of Alzheimer's disease for varies of reasons [4]. However, some recent research showed that the polarization state of microglia is reversible, offering a new perspective for treatment. Gamma frequency, a type of brain oscillation closely associated with cognitive functions, has been found to be impaired in AD patients. Recent studies suggest that modulating gamma frequency through non-invasive brain stimulation techniques may facilitate the shift of microglia from the M1 to the M2 phenotype, thereby slowing the pathological progression of AD [5]. This research proposal aims to explore this frontier field, investigating whether specialized modulation of gamma frequency can regulate the polarization state of microglia, with the hope to develop therapeutic strategies for AD.

# 2. Methods:

## 2.1 Experimental Subjects and Grouping:

The study will utilize two mouse models: Wild-type (WT) mice serving as a normal control group, and 5xFAD mice as the Alzheimer's disease model group [6]. Gamma stimulation will be given to these two groups of mice, so at last there will be 4 experimental groups: WT control group with no stimulation, WT group with gamma stimulation, 5xFAD group with no stimulation, and 5xFAD group with stimulation. Each group will consist of at least four mice, with the number potentially adjusted based on the required precision of the experiment. After the experiment is completed, the data from the 4 groups will be compared.

## 2.2 Experimental Design:

Gamma wave stimulation will be conducted through visual stimulation protocol for one hour per day over a period of four weeks [7]. A 40Hz bulb will be used to stimulate the mice groups to modulate their brain's endogenous oscillatory activity with a non-invasively stimulation to the mouse brain. [5,7]. Electroencephalography will be used then to determine whether if the gamma wave frequency changed as planned. If the first method of stimulation is ineffective, alternative plan will be implemented: Transcranial alternating current stimulation(tACS) equipment will apply current to the mice's brain, thus cause a change in gamma wave frequency. Another thing needs to be ensured is the two control groups are handled similarly but without gamma stimulation.

## 1.1 Behavioral Testing and Sample Collection:

A series of behavioral tests will be conducted on the mice at the baseline (before the experiment begins), at the midpoint of the experiment (two weeks), and at the endpoint of the experiment (four weeks) to assess changes in spatial learning, memory, and working memory. At the same time sample collections will be conducted on the mice, in order to prepare samples for later data analysis. The measurements include conducting initial behavioral tests to assess baseline cognitive function. Behavioral tests in the study will be Morris Water Maze, assesses spatial learning and memory [8]; and Y-Maze, tests spontaneous alternation behavior to measure working memory [9].

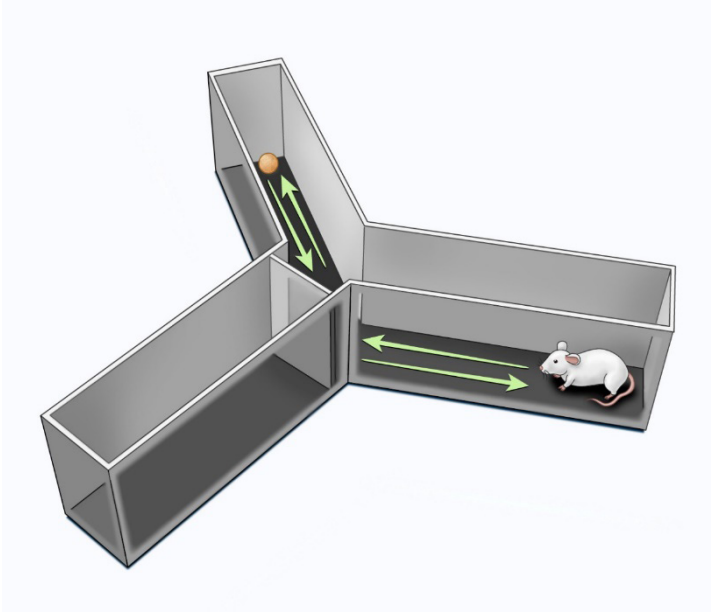

**Figure2. Example of Y-Maze**

In each assessment, the behavioral tests will be repeated. The aim of sample collection is collecting brain samples from a subset of mice to determine microglial activation through laboratory analysis. After the endpoint assessment, remaining mice will be euthanized.

### 2.3 Data Analysis:

The analysis of microglial activation will be completed by related methods of immunohistochemistry. First step is to fix the cells or tissue with a suitable fixative to preserve the antigen structure. Then the next step is incubating the samples with primary antibodies specific to M1 markers (e.g. CD86) and M2 markers (e.g. CD206) separately [3,10], which is the primary antibody incubation. This step allows the primary antibodies to bind to their respective targets which is M1 and M2 microglia. The next step is secondary antibody incubation: Incubate the samples with secondary antibodies conjugated to fluorophores (e.g. anti-rabbit FITC for M1, anti-mouse Alexa Fluor 594 for M2) that specifically recognize the host species of the primary antibodies [11]. Next, the brain sections will be analyzed by using a fluorescence microscope so that M1 and M2 microglia will be detected. After the laboratory analysis, the study aims to quantify the ratio of M1 to M2 microglia in each group of mice, and the ratios of control groups and experimental groups will be compared

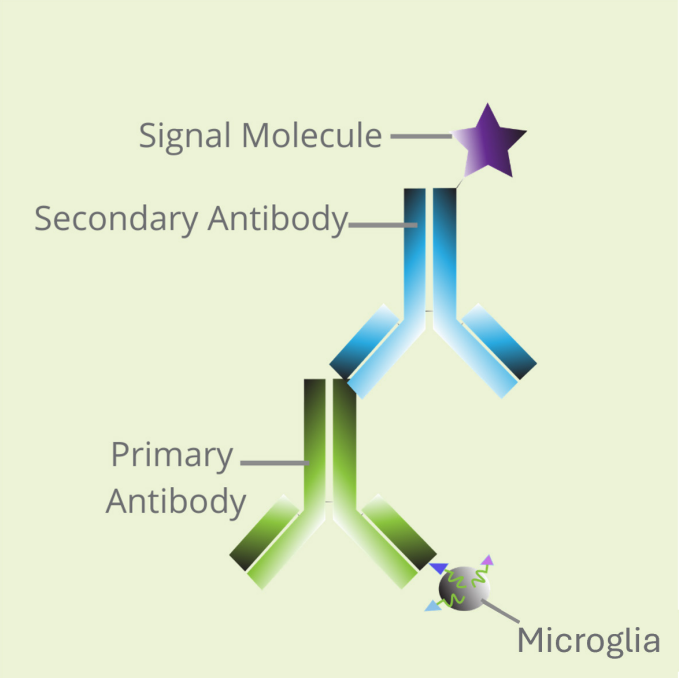


**Figure3. Demonstration of the principle of immunohistochemical methods**

## 3. Predicted results:

According to the principles of this study and by analyzing previous experiments, the prediction of microglial activation and cognitive function of mice can be given reasonable: The amount of M2 microglia identified will increase with the progress of experiment, and the proportion of M1 microglia will be expected to be significantly lower. It will lead to a trustworthy result that 5xFAD mice will improve their cognitive expression in Morris Water Maze and Y-Maze tests to reflect better memory retention and spatial learning ability, and in an ideal situation the cognitive function of 5xFAD mice will approach that of the control group mice. In general, the predicted result of experimental procedure is that Gamma wave stimulation will shift microglial activation towards an M2(anti-inflammatory) state and will improve cognitive performance in 5xFAD mice. These expected results will provide important clues for the potential of gamma wave stimulation as a non-invasive treatment to change the course of Alzheimer's disease and improve the quality of life of patients.

## 4. Discussion:

The study will conduct a comprehensive analysis of the impact of gamma waves on the state of microglia, particularly determine whether it can improve the transition from the pro-inflammatory M1 type to the anti-inflammatory M2 type, which is crucial for reducing neuroinflammation like Alzheimer's Disease. The advantages of this experimental procedure can be included as comprehensiveness and foresight. The study will examine the effects of

modulated gamma waves on Alzheimer's disease by both quantifying the transition of microglia from M1 to M2 and testing the cognitive function of mice groups, so that the whole experiment is comprehensive enough to ensure the accuracy of the results. On the other hand, the gamma wave stimulation method used in this study is innovative in the treatment of Alzheimer's disease, offering a fresh non-invasive treatment solution; the research is on the brink of uncovering key elements of Alzheimer's disease pathology by examining the activation of microglial cells and their impact on cognitive abilities, providing a sufficiently cutting-edge viewpoint.

Yet, there are caveats. The mice models employed in the experiment may not fully reflect the intricacies of Alzheimer's disease in humans, and the study's timeframe may be too short and brief to uncover the full scope of gamma wave treatments, including some long-lasting impacts and potential side effects. Furthermore, although this experimental procedure contains assessments as Y-Maze and Morris Water Maze, the experiment may not paint the whole picture of the improvement of cognitive functions. Nevertheless, the study's pioneering methods and thorough evaluation offer a fresh outlook, bringing a glimmer of hope for novel Alzheimer's therapies.

## 5. Conclusion:

This research proposal aims to provide a new perspective of understanding in Alzheimer's disease. To be specific, the cutting-edge progress is that it explores the impact of gamma wave stimulation on the transitions in microglial states, and fills the research gap between gamma wave stimulation, microglial activation states, and cognitive function. The experimental procedure may also make a contribution which lay the groundwork for the creation of non-invasive therapies based on gamma waves. In an ideal situation, this research will promote the process of slowing down in the progression of Alzheimer's disease and enhance the quality of life for patients undergoing treatment.

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