

# Activation of Microglia and Attenuation of Amyloid Beta Protein in Hippocampus Through Sound Induced 40Hz Gamma Oscillation

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**Abstract:** Alzheimer's disease (AD) has been a problem for many aged people but the pathogenesis hasn't been fully understood yet. The ways to treat it which are found at present are not quite effective. As the AD causes huge loss to the society each year, it is crucial to develop a new way using sound wave to treat it. A new way of clearing the amyloid beta protein may help solve this problem and lead the study of AD to a new road. A type of immune cell called microglia cell, which was found in central nervous system, has the ability to clean Amyloid beta protein through phagocytosis. This can be a better way to clear the amyloid beta protein by activation of microglia. Thus, we build a new method for inducing 40Hz gamma oscillation through clicking sound re is a new way of activating the microglia cell, which is safer and more convenient. So, using sounds to solve the problem is considered.

## 1 INTRODUCTION

Amyloid beta protein has been greatly studied in AD and scientists have tried to find a way to clear it as it is found in AD patients' brain and accumulate more as the disease getting worse. So, it is reckoned that clearing the amyloid beta protein would help cure the AD. To better clear the amyloid beta protein, they for AD therapy in 2012 and 2021, respectively. However, the results weren't so perfect as the symptom of the AD patients, such as memory loss, showed no remission (Dennis Thompson, Healthday Reporter).

In a former study the relationship between gamma oscillation and microglia cell activation was studied. 40Hz gamma oscillation is proved to have the ability to activate the microglia cell (Martorell, Paulson, Suk et al 2019). However, the study included invading or complicated process of inserting electric rods into the mice brains and light flicker and we hope to look for a way which is more safe and convenient. Thus, the use of sound is considered. Sound treatment is a way which does not invade the organs. Because the use of light has been accepted in this type of study, we think of using another kind of senses for inducing 40Hz gamma oscillation (Hardy, Higgins 1992).

Microglia cell is a kind of neuroglial cell and it has the ability to degrade the useless protein or other

structures in the brain tissue. After being stimulated, the microglia cell can be activated and start to clear the Amyloid beta protein with higher efficiency. In this test we will find out how to stimulate it and if it would work.

The Amyloid beta(A $\beta$ ) protein is largely found in AD patients' brain, and Amyloid beta hypothesis is now mainly accepted as pathogenesis of AD. The amyloid beta protein is closely related to the AD and clearing the amyloid beta protein might have the ability to cure the AD. The monoclonal antibody of Amyloid beta protein, Bapineuzumab and Donanemab, have been applied in clinical study to activate microglia for Ab clearance.

Changes in gamma oscillations (20–50 Hz) have been observed in several neurological disorders. Furthermore, 40Hz oscillations induced by ontogenetic method in hippocampus has been well proved to reduce Amyloid beta through microglia activation.

In this passage, we aim to stimulate the gamma oscillation by sound waves with different frequencies and the influence gamma wave has on the clearing ability of the microglia cell. Then the microglia cell will be examined to see if it is activated and the amyloid beta protein is checked to find out if it had been cleared. If the microglia cell is stimulated but the Abeta protein isn't less, we may need to make

further assumptions. Also, Abeta is less but microglia isn't stimulated can happen as well.

In this situation we will need to find out the relationship between sounds and Abeta protein's relationship apart from microglia cell (Iaccarino, Singer, Martorell, et al, Healthday Reporter 2019).

## 2 EXPERIMENT APPROACH

### 2.1 Experiment Materials and Equipment

30 5XFAD mice, divided into 5 groups, all female and 2 months old, with similar physical quality including weight and length; Amyloid beta 1–40 or Amyloid beta 1–42 ELISA (Enzyme-Linked Immunosorbent Assays) kit (Invitrogen), mobile phone with APP for sound maker (The mobile phone is required to have the ability to make sounds clear enough)

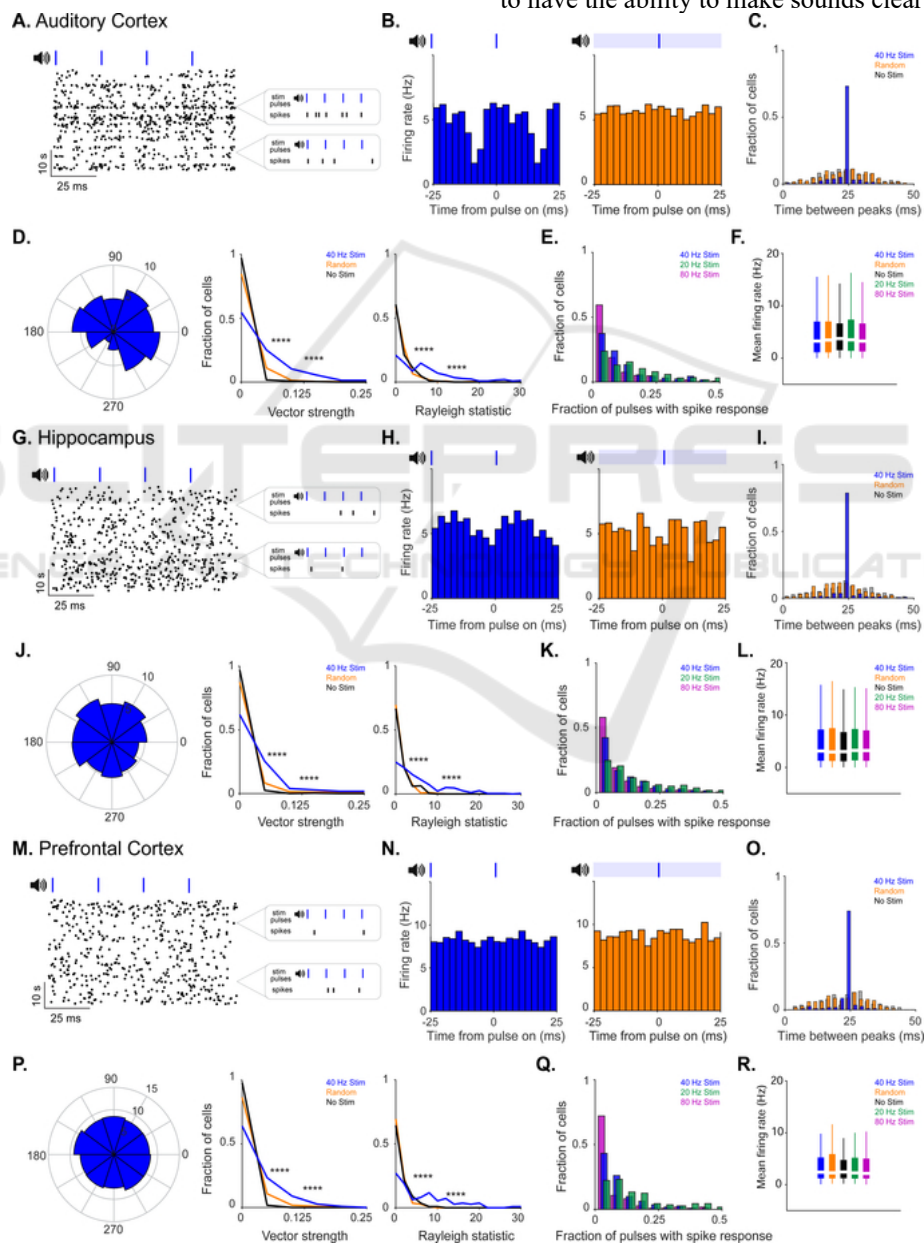


Figure 1: 40Hz gamma oscillation (Martorell, Paulson, Suk et al 2019).

Table 1: This is a sample figure for the experiment, showing the condition of the mice.

order number of mice/behavior types	sleeping time/h	food amount/g	times moving/h	frequency of fighting/times
1(20Hz)				
2(40Hz)				
3(60Hz)				
4(80Hz)				
5(no sounds)				

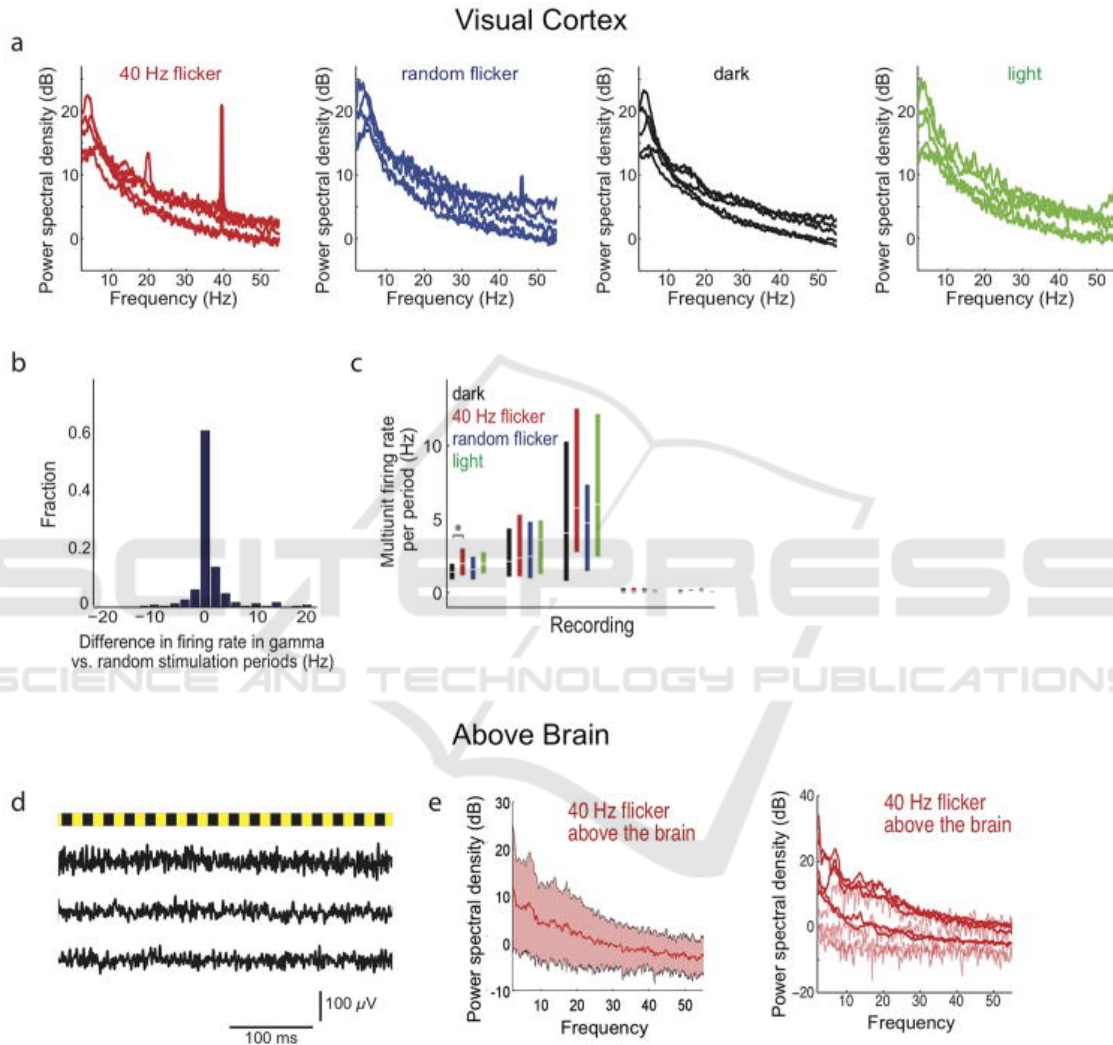


Figure 2: Visual cortex (Hardy, Higgins 1992).

Special made cages with sound proofing materials, making sure that the mice won't be disturbed by other sounds. The cage is large enough for the mice to move and water and food is provided 24 hours. The food and water amounts is calculate in the experiment.

The mice need a certain time to grow and have the accumulation of the amyloid beta Protein and two months is suitable. Also, a group of female mice is

easier to live together as they are less likely to fight with each other like male mice do. The male mice movements is also more difficult to observe as they are more likely to move there and here so we may ignore the change of mice behavior (Cerebral Cortex, Nicole Barger, Janet Keiter, Cerebral Cortex 2019).

## 2.2 Determination of the Effect of the Sound

First, we need to find out if the sound stimulation can be emerged in the mice brain including CA1 area and dentate gyrus area. Stimulation of sounds are given to the mice and the rate of firing is detected. The sounds are made while they are having food, sleeping or running on the spherical treadmill so that the mice conditions' influence is lowered. The test will include 20-100Hz sounds which would be used later in sound experiment. The test of signal will need the electric rod which will be put in to the hippocampus but in the later sections invading ways will not be used. In this section the effect of mice fighting against each other is also detected using male mice so that we could make sure the use of female mice is necessary. If the mice fighting will cause the change of gamma wave we would be able to find it out in this part of experiment.

## 2.3 Determination of Amyloid Beta Protein

30 5XFAD mice without any treatment are tested with Amyloid beta ELISA kit from nvitrogen to detect the original amount of amyloid beta protein. The CA1 region is isolated from mice, placed in the salty water, then CA1 tissue is lysed in PBS firstly to extract PBS (phosphate buffer saline) soluble Amyloid beta protein and lysed in guanidine hydrochloric acid to extract the insoluble Amyloid beta fraction. The soluble Amyloid beta protein fraction probably contained monomeric and oligomeric Amyloid beta protein. The Amyloid beta protein is then collected and determined by ELISA kit according to manufacture

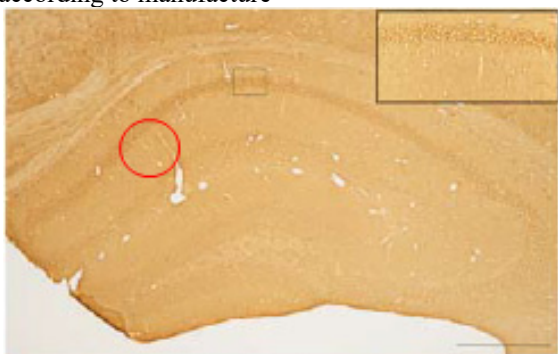


Figure 3: The area that we used to test the Amyloid beta protein (The Down-Expression of ACE and IDE Exacerbates Exogenous Amyloid-Neurotoxicity in CB2R-/- Mice).

instruction. The antibody from the ELISA kit is coated to the disc and the extra solution is washed off. Then the sample is added to it. The disc is washed and the process is repeated. At last the enzymes are put in to let it show the colour which can tell how much is there the amyloid beta protein.

Spectrometer can be used to test how much amyloid beta protein is found. The sample from mice and a sample of distilled water is tested at the same time to get rid off the effect of water refracting light. This will help making the result rational in the future (Julie Mazzolini, Sigrid Le Clerc, Glia Wiley 2019, Ana Badimon, Hayley J. Strasburger, Pinar Ayata, Nature. 2020).

## 2.4 Sound Experiment

The sound experiment lasts for 10 days. The sound is made by a mobile phone and certain app is downloaded which can make sounds of certain frequency. The cage will be covered with sound proofing materials to prevent extra sound influencing the experiment. The sound source is placed in the cage directly so the sound is clear enough. The sound source is specially protected with shelter as mice may move or damage it. The sound making process will be controlled remotely for the movement of sound source and opening of cages may cause influence on the mice. The source must be designed to minimized the effect, so it should be as small as possible.

The cage is also huge enough to decrease the influence of echo sound. During the sound experiment, each groups of mice receive a different frequency of sounds.

Group 1 receives a 20 Hz sound frequency, group 2 receives 40Hz, group 3 receives 60Hz, group 4 receives 80Hz and there's a group 5 which receives no sounds. The gamma oscillation of sound-treated mice is also detected to see which sound wave has the ability to activate the 40Hz gamma oscillation.

It is expected to see the best results in 40Hz sound wave's experiment. The sound wave tend to cause the gamma wave which has the same frequency.

The mice are exposed to the clicking sound for 2 hours each day during the xperiment. The living environment of the mice are as quiet as possible to prevent the noise influence. The cage will be covered with sound insulation materials to absorb the extra sound. The cage is also huge enough to decrease the influence of echo sound. (Hardy, Higgins 1992, Iaccarino, Singer, Martorell et al 2019, Healthday Reporter 2019)

After the sound experiment the amyloid beta protein concentration is tested again. The results will



show if the amyloid beta protein concentration decreased after the experiment.

## 2.5 Detection of Microglia Activation

The microglia activation in hippocampus is also detected 5 days after the 10 days' sound experiment. The cell diameter and the expression of microglial engulfment associated genes are tested in this section and detection method is totally following Hannah F's method. The rate of the microglia cell which are activated can show the stimulation ability of the gamma wave. High number and rate of microglia cell which are activated show that the gamma wave is effected for stimulating the microglia cell. To get better results, tissue from different part of brains should be tested. In this way we can find out if the location of the cell will have an effect on the stimulation signal.

After the test the rate of microglia cell which are stimulated is checked. Mean and standard deviation is calculated through the statistics and the numbers which are too high or low will not be chosen as a reliable result according to the results quartile number (both Q1 and Q3).

For the mice, the sound could be some kind of noise and cause damage to their body. During the test the heart rate, blood pressure and body temperature of the mice is also tested. The effect that the experiment had on the mice can not be estimated easily and these test could help us to find out. If the physical quality of the mice changed too much then the experiment will be less precised for when don't know if the microglia cell is stimulated by the sound or the change of body situation.

After the experiment, the movement and motion of the mice is also reserved to see if the mice moving ability has changed. The sound may cause the insanity of the mice, which would be dangerous for human patients.

For the observation of the mice, many parts needed to be taken care of. The basic observation include the sleeping time of the mice, the amounts of food the mice had and the times of specific behavior such as clearing the fur. For other actions such as the probability of running, they are calculated in the event of the mice has obvious changes which may result from the test.

As the number of mice isn't too large (number<30), Student's t test is also used. However, because the result might not be a Normal distribution the result may not be precised. The mice are divided in to groups and Student's t test, therefore can test if the mice stimulated microglia cell rate is different

between different groups. ( $H_0$ :rate in group 1=rate in group 2, $H_1$ :rate in group 1 $\neq$ rate in group 2,using two-sided test and see if  $H_0$  is rejected.)

## 2.6 Detection of Amyloid Beta Protein Clearance by Microglia Cell

Then the amyloid beta protein is tested its location to see if the protein is cleared. Detection of microglia phagocytosis of Amyloid beta protein though co-localization of microglia and anti-Amyloid beta antibody is processed. The antibody is connected to fluorescent protein and we look for the place where fluorescence appears and that's the place where the protein goes.

## 3 EXPECTED RESULTS AND ANALYSIS

It's estimated that with different frequencies of sound waves, the mice are found with different gamma waves. The mice receiving 40Hz sounds are discovered to have 40Hz gamma wave. With this result, we can suggest that the 40Hz sound can cause he 40Hz gamma oscillation. The 20Hz sounds and 60Hz sounds has less influence, while the 80Hz sounds has the least effect to cause 40Hz gamma oscillation. Generally the relationship between the sound wave frequency and gamma wave frequency is estimated to be liner.

The result is expected to see the decrease of amyloid beta protein and the activation of microglia cell. The microglia cell will show a result of having larger diameter and a hange on engulfment associated genes expression, which shows that it is activated. The results that the amyloid beta protein was inside the microglia cell should also be detected. If so, then it proves that the microglia cell can be stimulated by the gamma wave caused by the sound. Thus, we find another way of clearing up the Amyloid beta protein with microglia cells' cleaning function.

However, there can also be results with the microglia cell not activated but the 40Hz gamma wave is detected. This could be caused by a damage in the brain caused by AD which breaks the link with microglia cell and other hippocampus cells. Safety Attention:

- 1.do not touch the reagent during the experiment
- 2.do not eat and drink or smoke or using make-up during the experiment
- 3.the reagent should be preserved carefully
- 4.to get the reagent avoid using metal equipment

5.the reagent should be attached with disposable dropper

6.the reagent shouldn't be exposed to the air as it can deteriorate

7.after the experiment the remains should be managed with care

The safety attention must be followed in the experiment because they will influence the accuracy of the result and the statistics.

## 4 CONCLUSION

The experiment can push forward the study of amyloid beta protein and the AD. The AD has been asking for a better therapy for a long time. The use of sound is both safe and convenient, as it only needs a way that requires little equipment and funding. This experiment gives us a new idea of clearing the amyloid beta protein.

Although the experiment is quite inaccurate, for n the experiment the sound wave which is 45Hz hasn't be considered and the result is not rational, it does make an approach to the solution to AD.

In the future, if this method is improved to be more effective, the new way of curing D may be simply let the patients into a sound giving room and let them stay for a few days. The patients do not need to pass the painful course of the treatment, the side effect and the family can save time and money. However, there's still much to we need to explore in the future: how the microglia cell was stimulated is still a question. We might find the answers in the future.

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