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Gamma Wave Stimulation Affects BACE1 Levels and Promotes the Transition of Microglia from M1 State to M2 State

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Abstract:

Alzheimer's disease (AD) is a devastating neurodegenerative disorder and it presents with the hallmark neuropathological features of amyloid-beta (Aβ) plaques, tau tangles and active neuroinflammation. Beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1), a key mediator of increased Aβ, has been identified as the primary enzymatic source for production of this pathologic peptide while reactive microglial cells contribute to ongoing neurodegeneration perhaps through activation into their aggressive M1 state. The present study endeavors for the first time to address how gamma wave stimulation modulates BACE1 and microglial polarization (from M1 to beneficial M2) in different AD models. We hypothesize that gamma wave stimulation will reduce BACE1, decrease Aβ plaque burden and shift microglials towards M2 phenotype leading to reduced neuroinflammation and maintain cognitive function. This study will target mouse models of AD which have undergone gamma wave stimulation and its effects on BACE1 expression, microglial polarisation markers, Aβ plaque load and cognitive performance. Hypotheses the treatment will reduce BACE1 activity, decrease M1/M2 microglial ratio, improve cognitive performance and delay Aβ plaque accumulation These data suggest promising therapeutic strategies for neuroinflammation and amyloidogenesis in AD.

Keywords: Alzheimer's disease; BACE1; amyloid-beta; microglia; gamma wave stimulation; neuroinflammation; M1/M2 polarization; cognitive function; Aβ plaques.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder that leads to multiple cognitive declines. It is characterized by the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles in the brain.[1] The production of Aβ peptides is primarily catalyzed by β-site amyloid precursor protein cleaving enzyme 1 (BACE1), an aspartyl protease that is targeted to mitigate the formation of Aβ plaques[2]. Elevated BACE1 activity is strongly associated with increased Aβ production, and consequently, the progression of AD[3].

Microglia play an essential role in neuroinflammation and the homeostatic regulation of the brain's microenvironment. In AD, microglia can manifest as various functional states, which can be classified into two major groups: the pro-inflammatory M1 state and the anti-inflammatory/tissue-repairing M2 state. Neuroinflammation is greatly promoted by M1 state microglia which stimulate neurodegeneration through releasing pro- inflammatory cytokines and other substances that are toxic to neurons. M2 microglia are involved in anti-inflammation, tissue repair, and the clearance of Aβ[4]. The change from M1 to M2 microglia phenotype might evoke positive responses in AD, resulting in neuroprotection and slowing down AD progression [5].

A potential therapeutic approach for AD in which brain oscillations are made up of gamma waves at around 40 Hz is stimulated by neuromodulation. It has been shown that Aβ levels can be reduced, synaptic function increased and cognitive performance improved in animal models of AD using gamma wave stimulation [6]. However, the exact ways through which this treatment influences the brain activities still remain ambiguous, although microglial activity modulation and BACE1 level alterations might have a key contribution to it. More specifically, M1 into M2 change may be facilitated by gamma wave stimulation thereby leading to a more neuroprotective environment hence reducing Aβ buildup [7].

The main objective of this research is to investigate the effect of gamma wave stimulation on BACE1 expression and activity and how could this affect the function of microglia in Alzheimer's disease. We hope that studying

these pathways would allow us to identify whether gamma wave stimulation can modulate microglia phenotype, thus reducing neuroinflammation and delaying AD progression. These results may provide new directions for interventions that target neuroinflammatory and amyloidogenic processes by giving a more in-depth understanding of the relationship between gamma wave stimulation, BACE1 regulation and the transition of microglial state.

Specifically, we will examine if BACE1 levels are reduced by gamma wave stimulation, which causes a subsequent decrease in Aβ production. Additionally, we will also explore whether gamma wave stimulation shift microglial phenotype from a pro-inflammatory M1 state to an anti-inflammatory M2 state.

2. Methods

2.1 Study Designs and the Experiment Groups

We are planning to conduct experiments using both laboratory and animal testing methods to investigate how gamma wave stimulation affects BACE1 levels and the transition of states. In the laboratory experiments we will expose microglia to an environment that promotes amyloid formation. In the animal testing phase, we will utilize the 5xFAD mouse model [8]. This study will involve three groups; a control group, without treatment a group treated with amyloid beta and a group treated with both amyloid beta and gamma wave stimulation.

2.2 In Vitro Study: Microglial Culturing and Gamma Wave Stimulation

In our laboratory experiment we plan to extract cells from young mice and cultivate them in standard conditions. To trigger an M1 inflammatory response we will expose the cells to Aβ42 oligomers at a concentration of 2 μM, for a duration of 24 hours. Following the Aβ treatment a specific group will receive gamma wave stimulation through a custom-made device that emits 40 Hz pulses for an hour over a span of 5 weeks. Post stimulation we will analyze BACE1 levels using blotting techniques. The polarization of microglia will then be assessed by examining M1 markers like iNOS and M2 markers such, as Arg1 using qPCR and immunocytochemistry methods.

2.3 In Vivo Study: the 5xFAD Mouse Model and Gamma Wave Stimulation

As for the in vivo study, the 5xFAD mice will be divided into three groups: untreated, treated with Aβ, treated with Aβ and gamma wave stimulation. Following the experiment protocols established by Iaccarino et al. (2016), the mice in the gamma stimulation group will be exposed to daily 40 Hz auditory stimulation for 1 hour over a 5-week timespan. We will measure BACE1 levels in the hippocampus and cortex by using Western blotting, while the microglial activation states will be examined using immunohistochemistry by specific targeting for M1 and M2 markers and Aβ plaque levels will be assessed by using thioflavin-S staining.

2.4 Statistical Analysis

The experimental data will be analyzed using one-way ANOVA and post-hoc Tukey's tests to determine the differences between the groups.

3. Expected Results

3.1 In Vitro Results

Thus, we hypothesize that Aβ42 oligomers stimulate microglial BACE1 expression via a gamma wave stimulation-sensitive mechanism. We also predict a decrease in M1 state microglia markers (e.g., iNOS) and an increase in M2 state microglia markers Arginase 01. This therefore reflect a transition of microglial cells from an inflammatory to anti-inflammatory status. In addition, the predicted results will indicate that activation of gamma wave stimulation can modulate microglial activity and lower BACE1 in a certain extent. This would also demonstrate the predicted effect of gamma stimulation on decreased both neuroinflammation and amyloid pathology [9].

3.2 In Vivo Results

For the 5xFAD mouse model, we expect gamma wave stimulation to result in an extensive decrease of BACE1 levels compared with no treatment and Aβ-only treated groups. Furthermore, we expect to observe a decrease in levels of Aβ plaques with concomitant upregulation M2

microglial markers and decline in the expression M1 molecules as well. If these predictions are correct, it would support that gamma wave entrainment may not just be decreasing BACE1 levels but could also encourage a beneficial alteration in microglial activation states. Accordingly, repressing AD pathogenesis [6,7].

4. Predictions

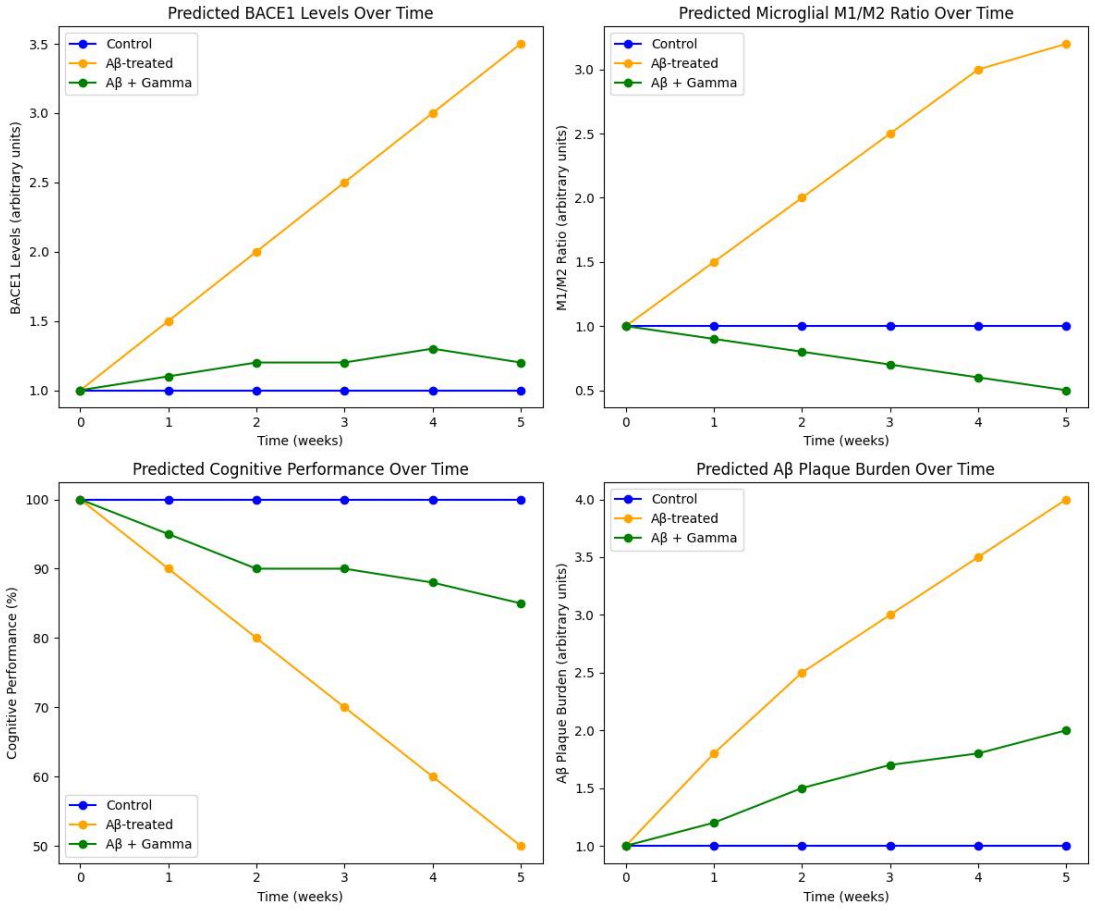

Fig.1.Experiment Result predictions

4.1 Predicted BACE1 Levels Over Time:

• The control group maintains stable BACE1 levels over time.

• The Aβ-treated group shows a continuous increase in BACE1 levels, indicating heightened amyloidogenic processing.

• The group receiving both Aβ and gamma stimulation shows a slight initial increase, but then tend to be stable, suggesting that gamma wave stimulation may inhibit the increase of BACE1 levels.

4.2 Predicted Microglial M1/M2 Ratio Over Time:

• We predict that the control group maintains a constant M1/M2 ratio, which indicates balanced microglial states.

• The Aβ-treated group shows a significant increase in the M1/M2 ratio over time, indicating a shift toward a pro-inflammatory (M1) state.

• The Aβ + Gamma group shows a decrease in the M1/M2 ratio over time, indicating a shift toward the anti-inflammatory (M2) state, suggesting that gamma wave stimulation can promote a neuroprotective microglial phenotype.

4.3 Predicted Cognitive Performance Over Time:

• The control group maintains regular cognitive performance.

• The Aβ-treated group shows a significant decline in cognitive performance over time, which reflects the cognitive harm on the mouse done by Alzheimer's disease.

• The group receiving both Aβ and Gamma wave stimulation shows a less steep decline in cognitive performance compared to others, which indicates that gamma wave stimulation may have a positive impact on controlling cognitive decline in AD.

4.4 Predicted Aβ Plaque Burden Over Time:

• The control group shows no significant change in Aβ plaque burden.
• The Aβ-treated group shows a continuous increase in Aβ plaque burden.
• The Aβ + Gamma group shows a much slower increase in plaque burden, suggesting that gamma wave stimulation reduces the accumulation of Aβ plaque.
Our predictions of the experiment result suggests that gamma wave stimulation could alleviate the damage done by AD by reducing BACE1 levels and promoting the M2 phenotype shift of microglia.

5. Discussion

Our study aims to research the effects of gamma wave stimulation on BACE1 levels and the transition of microglial state M1 to M2 in the context of Alzheimer's disease. We also hypothesize that gamma wave stimulation modulates neuroinflammatory processes and amyloidogenic pathways in AD. Therefore, we may offer a novel therapeutic approach to AD.

5.1 BACE1 Modulation and Amyloid Pathology

Amyloid-beta (Aβ) is generated by the essential enzyme β-site-cleavage enzyme 1 (BACE1). Activity of amyloid-beta is another critical point in suffering AD progression [2]. As we have hypothesized, a reduction in the levels of BACE1 caused by gamma wave stimulation would lead to the decreased production of Aβ. So, preventing Aβ plaques from forming. Earlier studies have shown that BACE1 levels are regulated by different forms of neural activity and neuroinflammation [3], such as those generated in the context of our gamma wave stimulation, suggesting additionally either a direct or indirect influence through entraining altered behavior on this level. These results, if valid, would add support to the notion that gamma wave stimulation could be a therapeutic aid psychosis caused by amyloid-beta accumulation in AD.

5.2 Microglial Activation and State Transition

Microglia is a key player in the response of brain to AD pathology. Microglia, for example, changing from M1 phenotype to the anti-inflammatory M2 one that is important in neuroprotection and clearing Aβ [5]. This advertising of the change diminishes neuroinflammation in brain and increases clearing amyloid-beta plaques from mind with a twist. Gamma wave stimulation has been shown to reduce pro-inflammatory cytokine levels and increases phagocytic activity in microglia as well [6]. It thus suggests that gamma waves may help to drive a beneficial microglial phenotype – the M2 state of activation, when employed in interventions for AD. The expected increase in M2 markers and the decrease in M1 markers would further prove that gamma wave stimulation serves as a modulator of microglial functioning.

5.3 Implications for Alzheimer's Disease Therapy

These findings may have implications for new non-invasive therapeutic strategies to treat AD in the future. However, current therapeutic strategies that forms the inhibition of BACE1 basically face a series of issues with deleterious effects and dubious efficacy [11]. Gamma wave stimulation presents a lighter choice, as it modulates by neural signals instead of pharmacological intervention BACE1 and microglial activity. In addition, the observation that gamma stimulation improved cognitive function in AD models [7] proposed not only to halt disease progression but also increase patients' quality of life.

5.4 Limitations and Future Directions

The exact mechanisms which gamma wave stimulation affects BACE1 and the microglia still remains unclear. Therefore, further research will be needed to explore these pathways. In addition, the problem of translating these results from AD mouse models to human patients still remains. Moreover, long-term effects of stimulation are yet to be discovered, leaving effects over time unknown.

6. Conclusion

This research presents a novel treatment of AD by studying the effects of gamma wave stimulation on BACE1 levels and microglial state transitions. In addition, this study fills the gap between the correlation of gamma wave stimulation and BACE1 levels and microglia activation states. It also will lay the foundation of gamma wave-based non-invasive treatments targeted to slow down the progression of AD while improving the quality of life for patients in treatment.

References

[1] De-Paula, V. J., Radanovic, M., Diniz, B. S., & Forlenza, O. V. (2012). Alzheimer's disease. Sub-Cellular Biochemistry, 65, 329–352.

[2] Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., ... Citron, M. (1999). β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science, 286(5440), 735–741.

[3] Cole, S. L., & Vassar, R. (2007). The role of amyloid precursor protein processing by BACE1, the β-secretase, in

Alzheimer disease pathophysiology. The Journal of Biological Chemistry, 282(4), 3142–3146.

[4] Cherry, J. D., Olschowka, J. A., & O'Banion, M. K. (2014). Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. Journal of Neuroinflammation, 11(1), 98.

[5] Hu, X., Li, P., Guo, Y., Wang, H., Leak, R. K., Chen, S., Gao, Y., & Chen, J. (2015). Microglia/Macrophage Polarization Dynamics Reveal Novel Mechanism of Injury Expansion after Focal Cerebral Ischemia. Stroke, 43(11), 3063-3070.

[6] Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., Mathys, H., Seo, J., Kritskiy, O., Abdurrob, F., Adaikkan, C., Canter, R. G., Rueda, R., Brown, E. N., Boyden, E. S., & Tsai, L. H. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature, 540(7632), 230–235.

[7] Martorell, A. J., Paulson, A. L., Suk, H. J., Abdurrob, F., Drummond, G. T., Guan, W., Young, J. Z., Kim, D. N. W., Kritskiy, O., Barker, S. J., Mangena, V., Prince, S. M., Brown, E. N., Chung, K., Boyden, E. S., & Tsai, L. H. (2019). Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. Cell, 177(2), 256–271.

[8] Oakley, H., Cole, S. L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., Berry, R., & Vassar, R. (2006). Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. Journal of Neuroscience, 26(37), 10129-10140.

[9] Adaikkan, C., Middleton, S. J., Marco, A., Pao, P. C., Mathys, H., Kim, D. N., Gao, F., Young, J. Z., Sohrabi, S., Hsieh, C. L., Mungenast, A. E., Rodriguez-Isiordia, C., Brock, J. A., Hemberg, M., Regehr, W. G., Boyden, E. S., & Tsai, L. H. (2019). Gamma Entrainment Binds Higher-Order Brain Regions and Offers Neuroprotection. Cell, 177(2), 256–271.e22.

[10] Hickman, S. E., Kingery, N. D., Ohsumi, T. K., Borowsky, M. L., Wang, L. C., Means, T. K., & El Khoury, J. (2008). The microglial sensome revealed by direct RNA sequencing. Nature Neuroscience, 13(5), 777–779.

[11] Egan, M. F., Kost, J., Tariot, P. N., Aisen, P. S., Cummings, J. L., Vellas, B., ... & Hilliard, A. A. (2018). Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. New England Journal of Medicine, 378(18), 1691-1703.