Neural Mechanisms Underlying Brain Gamma Entrainment after Periodic Auditory Stimulation at Gamma Frequency

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Abstract—**External sensory stimuli presented at gamma frequency have been shown to mitigate cognitive symptoms in Alzheimer's disease. However, the neural mechanisms underlying this therapeutic effect remain elusive. In this study, we investigated the effects of auditory stimulation at gamma frequency on brain oscillations and the role of theta oscillations in the process of entrainment in a group of dementia patients. Our findings indicate that external auditory stimulation at gamma frequency effectively produces gamma entrainment in the brain, and high theta power at rest predicts the level of gamma entrainment. These data suggest that external sensory stimulation modulates brain oscillations by enhancing gamma entrainment, particularly in patients with higher theta power. Our study sheds light on the potential future applications of non-invasive external sensory stimulation at gamma frequency in treating neurodegenerative diseases. However, the mechanisms underlying the generation of theta and gamma oscillations and their interactions are still not fully understood, and further research is needed to investigate these mechanisms and to develop targeted treatments that can enhance cognitive function by modulating brain oscillations. In summary, our study provides valuable insight into the potential therapeutic benefits of gamma frequency stimulation in the treatment of Alzheimer's disease and highlights the importance of understanding neural oscillations in the brain.**

Keywords—**Alzheimer's disease, gamma frequency, auditory stimulation, brain oscillations**

I. INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disease that is associated with various brain dysfunctions leading to dementia. The deteriorated state of the AD brain involves the loss of synapse, which is caused by the formation of insoluble plaque, amyloid-beta (Aβ), outside of the neurons and tangled phosphorylated tau inside the neurons [1–3]. As the loss of synapse advances, network operation starts to fail. The structure and function of the brain's neuronal network are affected by the disorder of the brain's rhythm and oscillation synchrony [4]. Inhibitory gamma-band activity is decreased, while excitatory neurons become more active in the disrupted network after AD, and this produces more Aβ, which further exacerbates the network's wellness [2, 5–11]. The disrupted network also affects cross-frequency coupling functions like theta-gamma couplings, which are important for memory and cognitive performance [4, 6, 12–14]. Areas such as the entorhinal cortex, the limbic system, and the hippocampus, which are known to be responsible for memory, cognitive, and sensory performance, exhibit clear gamma oscillation. However, these oscillations are disrupted in AD patients [6, 10, 15].

It is still unclear whether the network deficiency is the effect of the existing neuro dysfunctions or whether it is the cause. A report suggests that the synaptic loss caused by the surplus of Aβ leads to an increase in activity in the excitatory neurons, which leads to network disruption [2, 8]. However, other evidence shows that network disruption, such as reduced brain oscillation, occurred before the loss of synapses [16–18]. More recent evidence shows that the performance of the brain's oscillation affects memory and attention performance [19, 20]. Based on this evidence, new AD treatments based on non-invasive brain stimulations at gamma frequency are likely to improve network disruption and relieve the lowered quality of the brain's oscillation activity, in addition to conventional pharmacological treatments. To better understand the neural mechanisms underlying these methods, we examined whether gamma auditory stimulation can produce gamma entrainment in the brain and what determines the quality of the entrainment in this study.

II. METHODS

A. Data Description

Data of 13 people with memory complaints were retrieved from the "Auditory Gamma Entrainment" dataset on the OpenNeuro website (accession number: ds003800).

Participants in the dataset were tested and classified as non-AD or mild AD.

Each participant has their own folder labeled by their participant ID, which contains the Electroencephalography (EEG) data and metadata of the specific participant. Each participant may have their specific events during the task, and these along with the description of the task, channel names, and coordinates, are stored as metadata. The events are labeled with numbers indicating their sequence; number 1 represents the start of the resting trials and number 2 represents the start of the stimulus trials. The file contains rows and columns of the data where the rows represent information about the EEG at a specific time, and the columns show the EEG data of each specific channel.

The EEG data contains one resting task and the gamma auditory entrainment task, both performed with eyes open. The resting data was collected before the main task and consisted of a one-minute duration. The gamma auditory entrainment task, which is the main task, consists of six trials of stimulus and rest periods. The EEG data of the resting task and the main task are stored in separate files in each participant's folder.

B. Participants

The data used in the present study were retrieved from the OpenNeuro website. Cognitive abilities of the subjects were measured by the Mini Mental State Examination (MMSE). Participants had to be over the age of 55 and have no prior history of stroke, schizophrenia, traumatic brain injury, or major depressive disorders. Patients with diseases such as multi-system atrophy, progressive supranuclear palsy, Parkinson's disease, and cortico-basal degeneration were excluded from the study due to their potential effects on the brain. Participants who had received electroconvulsive therapy within the last six months were also excluded from the study. Participants with peak frequency amplitude of the response at the 40 Hz stimulant frequency at least three times the standard deviation away from the mean of response amplitudes in a range of adjacent frequencies (38 Hz to 42 Hz) after auditory stimulation were considered entrained (E), otherwise considered non-entrained (NE).

C. Task Execution

A one-minute resting state with eyes opened was measured to record the resting state potential for each participant. The auditory task was performed after measuring resting state potential and consisted of six trials of auditory stimuli with five resting trials in between. The auditory task involves 40 seconds of auditory stimuli followed immediately by 20 seconds of silence. The auditory stimuli were predesigned and played by two speakers 50 cm in front of the participants, with the speakers positioned 50 cm apart from each other. Outside factors were eliminated for the best results, and the participants would only be exposed to a volume of 40 dB while the room was kept quiet. Participants were required to sit comfortably with their eyes open while data was collected, and the harshness of the sound for each individual was considered for further tuning.

D. EEG Recording

The data were collected using 19 monopolar channels sampled at 250 Hz. Participants were instructed to relax their muscles to avoid muscle artifacts. The EEG cap was not removed even during the resting state and the recording was later separated into two parts.

E. Data Preprocessing

To eliminate none-target signals, we applied a 1 Hz high-pass filter and a 45–55 Hz notch filter. The filters eliminated any signal that is under 1 Hz and at 50 Hz. We rejected bad channels and averaged the two nearby channels to get an estimate of the rejected channels. We then averaged all the channels and subtracted them from individual channels to remove the common noise. We cleaned the data that was corrupted by motion artifacts.

F. Auditory Stimulation

There were six stimulus trials, with rest trials in between (20 seconds of silence). Each stimulus trial was a single 5 kHz sinusoid carrier with zero phase modulated with a zero-phase 40 Hz rectangular wave with 4% duty cycle (1 msec ON and 24 msec OFF cycles). Each trial lasted 40 seconds. All the trials, including the rest trials, were 340 seconds in total.

III. RESULTS

A. Correlation of General Information of the Patients and the Chance of Gamma Entrainment

To examine whether the chance of gamma entrainment is correlated with patients' general demographic information, we quantified various demographic parameters from patients entrained by gamma auditory stimulation and those not entrained. As shown in Fig. 1, we found that the Mini-mental State Examination (MMSE) values were similar between the non-entrained group (NE) and the entrained group (E) (NE: 23.86 ± 4.22, E: 25.00 ± 3.16, $n = 4, 7$; two-sample t-test, $p = 0.6512$) (Fig. 1(A)). Next, we examined the age distribution and found that the age was comparable between the two groups (NE: 75.71 ± 12.51 yr, E: 71.25 ± 4.79 yr, $n = 4, 7$; two-sample t-test, $p = 0.5179$) (Fig. 1(B)). Finally, we compared the number of patients entrained and non-entrained in males against females. In males, four patients were entrained and three were non-entrained, whereas in females all four patients were non-entrained. We conclude that the percent of entrained and non-entrained is similar for males and females (male $n = 4$ (E), 3 (NE), female $n = 4$ (NE); Fisher's exact test, $p = 0.1939$) (Fig. 1(C)). These data indicate that whether the patients can be entrained by gamma auditory stimulation is not relevant to their demographic information.
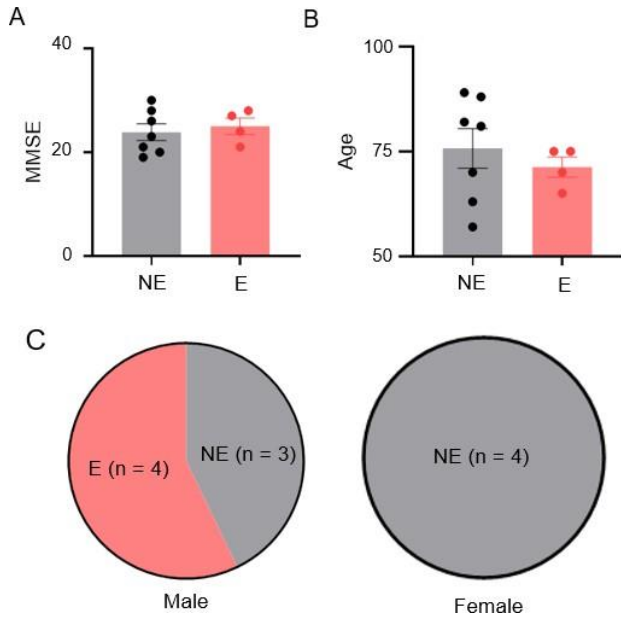
Fig. 1. Patient general information and gamma entrainment. (A) Bar graph of (MMSE) values of non-entrained (NE) versus entrained (E) patients. (B) Bar graph of Age of NE versus E patients. (C) Pie graph of NE and E cases in male and female patients. N.S., not significant. Error bar = Standard error of the mean.

B. Comparisons of Brain Activities during Rest Trials and Stimulus Trials

To examine the difference in brain activity between the resting trials and stimulus trials, we compared the statistics for the two trials. We first examined the normalized theta and gamma power to identify the change in brain activity between entrained and non-entrained patients during the auditory stimulus. Normalized power is calculated by taking the power of stimulus trials and dividing it by the power of resting trials. As shown in Fig. 2, we compared the normalized theta power and found that it is similar for non-entrained and entrained patients (NE: 1.34 ± 0.31, E: 1.53 ± 0.06, $n = 4, 7$; two-sample t-test, $p = 0.3143$) (Fig. 2(A)). We then compared the normalized gamma power and concluded that the normalized gamma power of entrained is higher than non-entrained (NE: 1.32 ± 0.17, E: 3.79 ± 0.63, $n = 4, 7$; two-sample t-test, $p = < 0.0001$) (Fig. 2(B)). To further analyze, we separated the rest and stimulus trials and examined each of them. For the theta power during stimulus trials, there is a higher theta power of entrained than non-entrained (NE: 0.30 ± 0.06, E: 0.61 ± 0.15, $n = 4, 7$; two-sample t-test, $p = 0.001$) (Fig. 2(C)). We then examined the theta power during resting trials and found that the theta power of entrained is higher than non-entrained (NE: 0.36 ± 0.10, E: 0.85 ± 0.04, $n = 4, 7$; two-sample t-test, $p = 0.0001$) (Fig. 2(D)). The individual theta power showed that entrained patients have higher baselines for both resting and stimulus trials, resulting in similar normalized theta power compared to the non-entrained patients. Finally, we compared the gamma power of entrained and non-entrained during stimulus and rest trials. We found that the gamma power of entrained and non-entrained during stimulus trials is similar (NE: 1.04 ± 0.28, E: 1.14 ± 0.18, $n = 4, 7$; two-sample t-test, $p = 0.5172$) (Fig. 2(E)). For the resting trials, the gamma

power is higher for the non-entrained than entrained (NE: 1.51 ± 0.47, E: 0.46 ± 0.13, $n = 4, 7$; two-sample t-test, $p = 0.0021$) (Fig. 2(F)). From the individual gamma powers, we conclude that the gamma power during stimulus trials is similar, but the gamma power during resting trials is higher for non-entrained patients, making the ratio between stimulus and resting trials higher for the entrained patients, which we can see from the normalized gamma power.
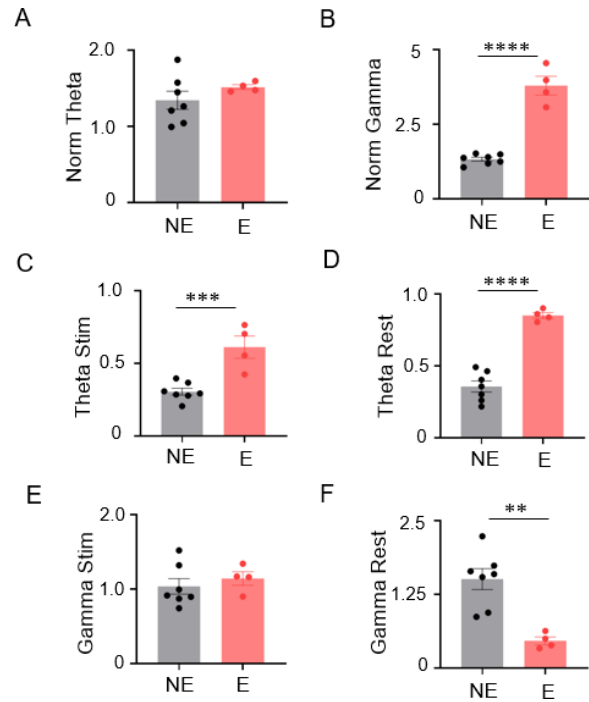

Fig. 2. Brain activity and gamma entrainment. (A) Bar graph of norm theta power of non-entrained (NE) versus entrained (E) patients. (B) Bar graph of norm gamma power of NE versus E patients. (C) Bar graph of theta power during stimulus trials of NE versus E patients. (D) Bar graph of theta power during rest trials of NE versus E patients. (E) Bar graph of gamma power during stimulus trials of NE versus E patients. (F) Bar graph of gamma power during rest trials of NE versus E patients. N.S., not significant. **: $p < 0.01$. ***: $p < 0.001$. ****: $p < 0.0001$. Error bar = Standard error of mean.

C. Frequency-Specific Effects after Gamma Entrainment

We then sought to study whether the entrainment of brain oscillations by gamma auditory stimulation was frequency specific. To address this question, in addition to the gamma frequency activity mentioned above, we further quantified the powers of alpha (8–12 Hz) and beta band activity (20–24 Hz) during stimulation and rest state and compared these values in the non-entrained versus entrained patients. Interestingly, we found that the alpha activity during stimulus was comparable in non-entrained patients and entrained patients ($n = 7, 4$; two-sample t-test, $p > 0.05$) (Fig. 3(A)). The alpha activity in the rest state was also similar between the two groups ($n = 7, 4$; two-sample t-test, $p > 0.05$) (Fig. 3(B)). Moreover, the beta activity during stimulus and in rest state was also similar in non-entrained patients and entrained patients ($n = 7, 4$; two-sample t-test, $p > 0.05$) (Fig. 4(A–B)). These data together indicated that the gamma auditory stimulation

specifically entrained brain oscillation at gamma frequency, leaving activities at other frequency unchanged.
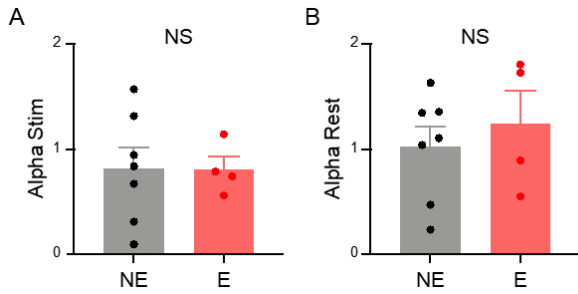

Fig. 3. Alpha band activity in patients. (A) Bar graph of alpha power during stimulus trials of NE versus E patients. (B) Bar graph of alpha power during rest trials of NE versus E patients. N.S., not significant. Error bar = Standard error of mean.
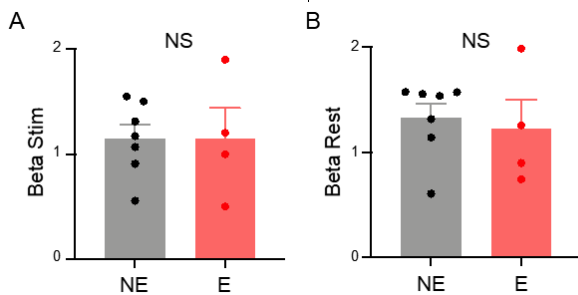

Fig. 4. Beta band activity in patients. (A) Bar graph of beta power during stimulus trials of NE versus E patients. (B) Bar graph of beta power during rest trials of NE versus E patients. N.S., not significant. Error bar = Standard error of mean.

IV. DISCUSSION

In the present study, we found that the external auditory stimulation at gamma frequency effectively produced gamma entrainment in the brain. Additionally, high theta power at the resting state predicted the level of gamma entrainment. Together, these data suggest that external sensory stimulation at gamma frequency can change brain oscillation by enhancing gamma entrainment, especially in patients with higher theta power brain activity in the resting state.

Synchronized oscillations are crucial and affect long-range communications in the brain [14, 21]. We already know some functions of the hippocampus, such as the generation of theta and gamma oscillations during sensory data binding and memory processes [22, 23]. However, the mechanism underlying the generating of these oscillations and their interactions are still being investigated. Interactions between excitatory pyramidal cells and inhibitory interneurons contribute to the gamma activity [4, 11, 14, 24]. Malfunctions in inhibitory signaling or loss of inhibitory synapses can result in arrhythmic brain oscillations [2]. Theta oscillations can also be caused by the inhibition of pyramidal cells by the upstream area in the hippocampus, which also modulates gamma activity [14, 24, 25]. In this study, we found that the entrained group during rest showed high theta power with low gamma activities. An increase in gamma power was associated with a decrease in theta power.

The role of theta oscillations in entertainment is to increase the intensity of entrainment. To justify this effect, we can consider the process of theta nested gamma oscillations which reflects the interaction of pyramidal cells and interneurons. This interaction involves feedback loops between excitatory and inhibitory populations in the hippocampus, through which pyramidal cells induce theta cycles in the interneurons, which in turn send inhibitory signals to the pyramidal cells as gamma cycles.

V. CONCLUSION

In this study, we investigated the effects of external auditory stimulation at gamma frequency on brain oscillations and the role of theta oscillations in the process of entrainment. Our findings suggest that this type of stimulation can effectively produce gamma entrainment in the brain and that higher theta power at the resting state predicts the level of gamma entrainment. Our results suggest that external sensory stimulation can change brain oscillations by enhancing gamma entrainment, especially in patients with higher theta power brain activity in the resting state.

However, the mechanisms underlying the generation of theta and gamma oscillations and their interactions are still not fully understood. Further research is needed to investigate the underlying mechanisms and to develop targeted treatments that can enhance cognitive function by modulating brain oscillations. In addition, the sample size of our study was relatively small, and future studies with larger sample sizes are necessary to confirm our results.

Despite these challenges, our study sheds light on the potential future applications of non-invasive external sensory stimulation at gamma frequency in treating neurodegenerative diseases. Our study also highlights the importance of synchronized oscillations in the brain and the role of theta oscillations in the process of entrainment. We hope that our findings will inspire further research in this area and contribute to the development of new treatments for neurological disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Haian Shao and Haiping Shao conceived of the presented idea, preformed the calculations, analyzed the data, worked on the technical details, and wrote the paper. All authors discussed results together and Weijia Wang proofread the paper. All authors had approved the final version.

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