Title: The Impact of Brain Auditory Stimulation in the Gamma Band on Cognitive Functions in Early-Stage Alzheimer's Patients

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To appear in: **Basic and Clinical Neuroscience**

Received date: 2025/06/30

Revised date: 2025/12/22

Accepted date: 2025/12/23

Please cite this article as:
Mehdizadeh Fanid, L., Beyrami, M., Jafari Asl, M., Nikzad, B., Dadashi, S., Zehtabi, Z., et al. (In Press). The Impact of Brain Auditory Stimulation in the Gamma Band on Cognitive Functions in Early-Stage Alzheimer’s Patients. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.7612.2
DOI: http://dx.doi.org/10.32598/bcn.2026.7612.2

Abstract

Alzheimer's disease (AD), a progressive neurodegenerative disorder marked by deterioration in cognitive abilities, currently affects an estimated 24 to 50 million individuals worldwide and lacks effective non-invasive therapeutic options. Disruption of gamma-band neural oscillations (~40 Hz), which are known to underlie higher-order cognitive processes such as working memory and executive function, has been repeatedly documented in both human and animal models of AD. Targeted auditory stimulation at gamma frequencies has recently emerged as a potential neuromodulatory strategy for mitigating these deficits. In a controlled experimental framework, thirty individuals clinically diagnosed with early-stage AD, confirmed via MRI and exhibiting Mini-Mental State Examination (MMSE) scores between 10 and 20, were divided into two groups. Thirty participants with early-stage Alzheimer's disease were enrolled and allocated into intervention (n = 15) and control (n = 15) groups using a quasi-experimental parallel-group pre–post design. The intervention group received 30 sessions of auditory stimulation using binaural beats centered at 40 Hz over the course of three weeks, while the control group listened to an acoustically comparable audio file lacking gamma-frequency modulation. Cognitive performance was assessed using computerized versions of the Corsi Block-Tapping Test, Wisconsin Card Sorting Test (WCST), and Digit Span Test. In addition, resting-state quantitative EEG (QEEG) was conducted to evaluate frontal lobe gamma-band activity under both eyes-open and eyes-closed conditions. Statistical analysis revealed that gamma-band auditory stimulation resulted in a significant enhancement of visuospatial working memory ($p = 0.001$, Cohen's $d = 1.42$) and cognitive flexibility ($p = 0.028$, $d = 0.78$), alongside marked increases in both absolute and relative gamma power in the frontal cortex under eyes-closed conditions ($p < 0.01$). These findings suggest that auditory stimulation at 40 Hz selectively modulates neural circuits associated with executive and spatial cognitive functions and may serve as a viable non-invasive adjunctive intervention in early-stage Alzheimer's disease.

Keywords: Gamma Band; Alzheimer's disease; Brain Auditory Stimulation; Working memory; Cognitive flexibility

1 INTRODUCTION

Dementia, characterized by cognitive impairment, impacts between 24 to 50 million people globally (Scheltens et al., 2016). Alzheimer's disease is the most common cause of dementia, constituting approximately 70 to 80 percent of cases. The primary features of the disease include the presence of extracellular beta-amyloid (Aβ) deposits and intracellular neurofibrillary tangles (tau) (Kumar et al., 2015). The affected demographic for Alzheimer's typically includes individuals over the age of 60, but it can increasingly affect younger individuals. Specifically, the prevalence of dementia in individuals under 50 years old is less than one in 4000 (Scheltens et al., 2016).

Alzheimer's is a multifactorial disease with a complex pathophysiology. Typically, the disease has an early preclinical stage. Identifying this early stage may serve as a crucial point for successful interventions (McDermott et al., 2018). Lifestyle-related factors such as diabetes, obesity, smoking, and depression have been highlighted as areas that, if modified, can reduce the risk of the disease (Scheltens et al., 2016).

Human EEG studies in Alzheimer's disease have reported heterogeneous gamma alterations depending on task demands, disease stage, and recording conditions. Therefore, gamma modulation should not be interpreted as uniformly deficient across all contexts. Research has shown that patients with Alzheimer's experience cognitive dysfunction (Lambon et al., 2003). These individuals demonstrate impaired performance in tasks related to planning, set shifting, inhibition, sustained attention, divided attention, and selective attention (Stopford et al., 2012). Aberrant neural activity can exacerbate the progression of Alzheimer's pathology, ultimately disrupting neural circuits involved in higher cognitive functions (Cantor et al., 2013). Conversely, neural activity can also be modulated to mitigate Alzheimer's pathology (Iaccarino et al., 2016). For instance, gamma oscillations (30-90 Hz), associated with multiple higher-level cognitive functions, have been disrupted in various Alzheimer's animal models (Gillespie et al., 2016; Iaccarino et al., 2016). Altered gamma, including reduced self-synchronization of gamma and diminished gamma power, has been observed in Alzheimer's patients and in several Alzheimer's animal models (Verret et al., 2012 & Gillespie et al., 2016).

Gamma oscillations can be induced through sensory stimuli, a phenomenon known as gamma synchronization (Iaccarino et al., 2016). The use of auditory sensory stimuli at a specific frequency can lead to synchronization of brain waves. Synchronization refers to the simultaneous rhythmic (or sequential) alignment to external events (Thut et al., 2011). Auditory stimuli at an optimal level for a short duration may serve as an auditory stimulus to activate various brain functions (Chaudhury et al., 2013). It is believed that the positive effects of auditory stimulation result from the activation of monoaminergic brain circuits, including serotonergic and dopaminergic pathways (Moraes et al., 2018). Additionally, auditory stimulation is hypothesized to facilitate neurogenesis in the hippocampus, promote neuronal reconstruction and repair by regulating the secretion of steroid hormones, ultimately leading to synaptic plasticity (Fukui et al., 2008).

Considering that studies have indicated a connection between neural activity in the gamma band and a wide range of cognitive functions (Herrmann et al., 2004), and concurrently, existing research literature suggests that individuals with Alzheimer's exhibit disrupted gamma activity and cognitive deficits, the central inquiry of this study

is whether brain auditory stimulation in the gamma band influences cognitive functions in individuals in the early stages of Alzheimer's disease. The present study builds upon our previously published work, which investigated the cognitive effects of 40 Hz auditory stimulation in patients with early-stage Alzheimer's disease (Mehdizadeh Fanid et al., 2025). While the prior study employed a single-group pre–post design focusing exclusively on behavioral outcomes, the current investigation extends this work by incorporating a parallel control group and quantitative EEG measures to examine neurophysiological correlates of the intervention.

2 METHODOLOGY

2.1 Data Collection

This study employed a quasi-experimental parallel-group pre–post design. Thirty patients diagnosed with early-stage Alzheimer's disease reside in Tabriz. were allocated into an intervention group (n = 15) and a control group (n = 15) using convenience sampling followed by group matching based on age, sex, and baseline MMSE scores. Using targeted sampling, 15 patients were recruited based on a neurologist-confirmed diagnosis consistent with the criteria outlined by the Alzheimer's Association-National Institute on Aging (AANIA). Inclusion required a definitive diagnosis of Alzheimer's disease, while exclusion criteria encompassed lack of consent, diagnoses inconsistent with Alzheimer's, reversible dementias such as hypothyroidism and substance-related conditions, severe depression, major organ failure, intracranial lesions, history of subdural hematoma or traumatic brain injury, infections including viral encephalitis, and other non-Alzheimer's dementias such as frontotemporal and Lewy body dementia. Ethical approval was granted by the University of Tabriz Ethics Committee (IR.TABRIZU.REC.1400.024), with informed consent obtained from all participants or their legal representatives. Following referral, participants underwent cognitive screening with the Mini-Mental State Examination (MMSE), and only those scoring between 10 and 20, indicative of moderate cognitive impairment, were included. Baseline assessments comprised the Computerized Digit Span Test for verbal working memory, the Computerized Corsi Block-Tapping Task for visuospatial working memory, the Computerized Wisconsin Card Sorting Test (WCST) for abstract reasoning and cognitive flexibility, and quantitative EEG (QEEG) recordings targeting gamma-band brain activity. Individuals presenting psychiatric comorbidities, reversible dementia causes, or histories of traumatic brain injury were excluded. The intervention involved auditory gamma-band stimulation administered over 30 sessions across three consecutive weeks, delivered daily at a fixed daytime schedule to control for circadian effects. Stimulation took place in a quiet, well-lit environment with participants seated comfortably and monitored throughout. Auditory stimuli were generated using Gnaural software (version 2.3.8), producing binaural beats at 40 Hz based on a 440 Hz carrier frequency with a 480 Hz offset, yielding a gamma-band modulation. Sound was delivered via calibrated Sparkle stereo headphones (Model SPK-X4) at approximately 60 dB. The control condition consisted of an audio stimulus matched to the intervention in duration and sound intensity but lacking structured interaural phase modulation at 40 Hz. While the control stimulus did not include rhythmic gamma-frequency binaural modulation, we acknowledge that broadband acoustic signals may contain energy across a wide frequency spectrum. Therefore, the control condition was designed to control for general auditory exposure rather

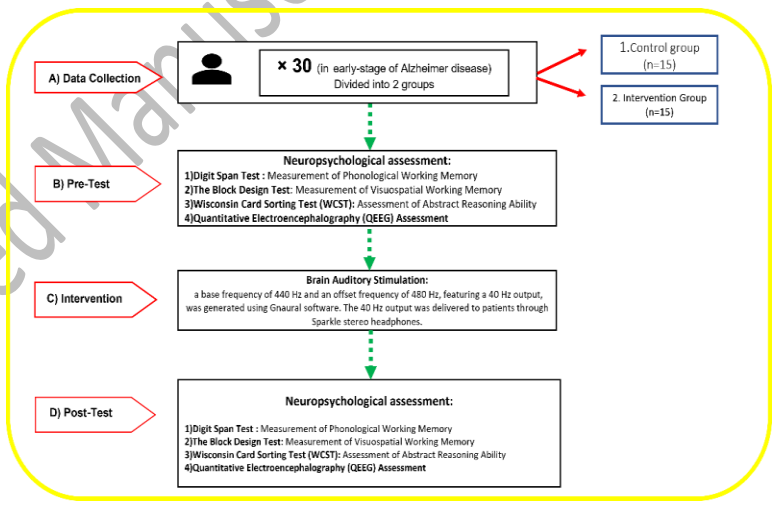
than to be spectrally silent in the gamma range. Accordingly, the critical distinction between the intervention and control conditions lies in the presence versus absence of structured rhythmic gamma-frequency entrainment rather than absolute gamma-band acoustic energy.

The carrier frequency of 440 Hz was selected because it lies within a comfortable and easily perceivable auditory range for most adults, minimizing listener fatigue and auditory discomfort (Reedijk et al., 2015). A frequency offset of 480 Hz produces a 40 Hz binaural beat (i.e., 480 Hz – 440 Hz = 40 Hz), which aligns with the gamma-band frequency range that has been associated with higher-order cognitive processing and sensory integration (Herrmann, 2001; Ross et al., 2014).

Although the optimal carrier and offset frequencies for inducing gamma-band entrainment in Alzheimer's disease have not been definitively established, previous auditory entrainment research in humans has reliably employed similar parameters to target gamma-range activity (Chaieb et al., 2015). Furthermore, studies investigating auditory steady-state responses suggest that binaural beat offsets near 40 Hz can elicit measurable gamma-band phase-locking in EEG recordings, supporting the rationale for this parameter choice (Galambos et al., 1981).

These selections represent a compromise between perceptual salience and the goal of engaging neural dynamics in the gamma range while avoiding excessive auditory strain in an elderly clinical population. Supervision ensured compliance, correct headphone placement, and absence of environmental distractions during all sessions. A subset of participants in the intervention group overlapped with those included in our previously published behavioral study; however, the present manuscript reports novel neurophysiological outcomes (QEEG) and includes an independent control group, allowing for a controlled comparison of intervention effects.

Assessments included the Wisconsin Card Sorting Test, Block-Tapping Test, Digit Span Test, and QEEG to evaluate gamma wave activity. Steps of the study are shown in Figure 1.



A. Neuropsychological assessment

1. **Digit Span Test**

This test serves to assess verbal working memory or cognitive digit span (MacLeod et al., 2008). In the computerized version of the Digit Span Test, participants encounter numbers presented both auditorily and visually. They are instructed to immediately replicate the presented numbers by inputting them using the keyboard. In the auditory-only rendition of the test, numbers are exclusively presented in an auditory manner, prompting participants for immediate repetition. The examiner records the responses into the computer. The sequence of presented numbers initiates with two digits and, with each accurate response, extends in length, progressing up to 9 digits. The test has demonstrated a reported reliability of 0.63 through the test-retest method (Piper et al., 2015).

2. **The Block Design Test**

The Block-Tapping Test, an adaptation of the Digit Span Test, focuses on assessing visuospatial working memory without verbal reliance. Despite the increasing complexity in sequences and stimuli, fMRI studies show consistent overall brain activity during the test, suggesting that encoding challenges do not significantly impact overall brain engagement (Toepper et al., 2010). The direct version of the test requires visuospatial working memory support, excluding the need for cognitive digit span. As the sequence length exceeds three or four, central executive resources become active (Vandierendonck et al., 2004). In this test, participants observe and memorize sequences of illuminated blocks, reproducing them by clicking on corresponding blocks. The test starts with two blocks and progressively increases in complexity up to nine blocks. It concludes that if two consecutive errors occur, it records the longest correctly recalled sequence. On average, individuals recall about five blocks, with a reported reliability of 0.73 in the test-retest method (Walker et al., 2010).

3. **Wisconsin Card Sorting Test (WCST)**

The WCST, devised by Grant and Berg (1948), assesses abstract reasoning through a set of 64 cards featuring symbols (triangle, star, cross, circle) in four colours (red, green, yellow, and blue). Participants match cards to an initial set, guided by specific characteristics deduced from examiner feedback. Following ten consecutive correct attempts, the principle shifts, indicating a category transition. Lazak's.

4. Quantitative electroencephalography (QEEG)

Resting-state quantitative EEG (QEEG) recordings were obtained from all participants before and after the intervention under two conditions: eyes open and eyes closed. Data acquisition was performed using the eWave EEG system (Pooyandegan Rah Saadat Co., Iran) with a high-pass filter set at 1 Hz, a low-pass filter at 70 Hz, and a sampling rate of 500 Hz. Electrodes were positioned according to the international 10–20 system, with particular emphasis on the frontal sites Fp1, Fp2, F3, F4, Fz, F7, and F8. To minimize contamination of gamma-band activity by muscle artifacts, EEG recordings were visually inspected, and channels with excessive noise were excluded. Independent component analysis (ICA) was applied to remove ocular and muscle-related components, with component rejection based on established spatial and temporal criteria. Subsequent quantitative analysis was conducted using NeuroGuide software, where absolute and relative gamma power within the 30–45 Hz frequency range were computed for each condition. Spectral analysis employed a Fast Fourier Transform (FFT) with a Hamming window and a two-second segment duration. Power values were

averaged across the specified frontal electrodes, and relative gamma power was normalized against the total power across all frequency bands to provide standardized measures of cortical activity.

3 RESULTS

To assess the impact of gamma band brain auditory stimulation on cognitive functions in Alzheimer's patients, the initial means of both experimental and control groups were compared before intervention using an independent t-test. The analysis revealed no significant difference between the group means before the intervention (p=0.884), suggesting a baseline homogeneity.

Subsequently, the post-intervention means of both groups were compared using an independent t-test. The mean for the experimental group after intervention was 48.87 (standard deviation: 2.72), while the mean for the control group after intervention was 46.60 (standard deviation: 3.06). The comparison of means using an independent t-test yielded a significant difference between the two groups (p=0.041). This indicates the effectiveness of gamma band brain auditory stimulation on the cognitive functions of Alzheimer's patients, resulting in an enhancement of cognitive performance in this patient cohort.

Table 1: Demographic Characteristics of Study Participants

		N (%)
Group	**Intervention Group**	15 (50)
	Control Group	15 (50)
Gender	**Man**	18 (60)
	Woman	12 (40)
Mean (SD)		
Age		60.93 (17.98)

To assess the efficacy of gamma band brain auditory stimulation on working memory for cognitive phonemic tasks in Alzheimer's patients, initial means of the experimental and control groups were compared before intervention using an independent t-test. No significant difference in means between the two groups was observed before intervention (p=0.058).

Subsequently, post-intervention means of both groups were compared using an independent t-test. The mean for the experimental group after intervention was 2.50 (standard deviation: 0.50), and the mean for the control group after intervention was 1.86 (standard deviation: 0.44). The comparison of means using an independent t-test revealed a significant difference between the two groups (p=0.001), indicating statistical significance in the observed mean difference. This suggests that brain auditory stimulation in the gamma band is effective in enhancing working memory for cognitive phonemic tasks in individuals with Alzheimer's disease.

In the context of investigating the impact of brain auditory stimulation in the gamma band on the absolute power of gamma waves in individuals with early-stage Alzheimer's, brainwave recordings were acquired in both open-eye and closed-eye conditions, with subsequent comparison of their absolute power in the gamma band within the frontal region. Initially, mean values of the two experimental and control groups were compared before the intervention using an independent t-test. No statistically significant differences in means between the two groups were observed before the intervention, with p-values of 0.933 in the open-eye condition and 0.082 in the closed-eye condition.

Following the intervention, post-intervention means of the two groups were examined using an independent t-test. The mean for the experimental group after intervention in the open-eye condition was 3.84 (standard deviation: 2.08), while the mean for the control group after intervention in the open-eye condition was 3.15 (standard deviation: 2.39). This difference was not statistically significant (p=0.408), suggesting that brain auditory stimulation in the gamma band did not significantly affect the absolute power of gamma waves in individuals with Alzheimer's in the open-eye condition.

In the closed-eye condition, the mean for the experimental group after intervention was 3.02 (standard deviation: 1.33), whereas in the control group, it was 1.47 (standard deviation: 0.69). The comparison of means using an independent t-test showed a significant difference (p<0.001), indicating that the application of brain auditory stimulation in the gamma band increased the absolute power of gamma waves in individuals with Alzheimer's in the closed-eye condition.

To explore the impact of brain auditory stimulation in the gamma band on the relative power of gamma waves in individuals with early-stage Alzheimer's, brainwave recordings were gathered under both open-eye and closed-eye conditions, focusing on the frontal region. Initially, mean values of the experimental and control groups were compared before the intervention using an independent t-test. No noteworthy difference in means was found before the intervention, with p-values of 0.170 in the open-eye condition and 0.053 in the closed-eye condition.

Following this, the post-intervention means of the two groups were assessed using an independent t-test. The mean for the experimental group after intervention in the open-eye condition was 2.15 (standard deviation: 1.45), and for the control group, it was 2.75 (standard deviation: 1.11). This disparity was not statistically significant (p=0.21), indicating that brain auditory stimulation in the gamma band did not significantly impact the relative power of gamma waves in individuals with Alzheimer's in the open-eye condition.

In contrast, for the closed-eye condition, the mean for the experimental group after intervention was 2.60 (standard deviation: 1.50), while for the control group, it was 4.29 (standard deviation: 1.44). The comparison of means using an independent t-test revealed a significant difference (p=0.004), signifying that the application of brain auditory stimulation in the gamma band effectively increased the relative power of gamma waves in individuals with Alzheimer's in the closed-eye condition. Further details can be found in Tables 2 and 3 in the subsequent section.

Table 2: Mean and Standard Deviation of Investigated Variables in Intervention and Control Groups before the Intervention.

VARIABLE	MEAN (STANDARD DEVIATION)		(P) VALUE
	Control Group	Intervention Group	
Cognitive Functions (Wisconsin Card Sorting Test)	45.80(2.542)	47.45(2.615)	**0.726**
Block-Tapping	1.96(0.39)	1.63(0.51)	**0.058**
Digit Span	2.13 (0.228)	2(0.387)	**0.252**
Absolute Gamma (Closed Eyes) Before	3.029 (2.353)	1.82 (1.04)	**0.119**
Absolute Gamma (Open Eyes) Before	2.72 (2.41)	2.65 (2.118)	**0.933**
Relative Gamma (Closed Eyes) Before	16.57(7.13)	12.44(6.766)	**0.11**
Relative Gamma (Open Eyes) Before	6.01(5.07)	8.87(6.07)	**0.17**

Table 3: Mean and Standard Deviation of Investigated Variables in Intervention and Control Groups after the Intervention.

Variable	Mean (Standard Deviation)		(P) value
	Control Group	Intervention Group	
Cognitive Functions (Wisconsin Card Sorting Test)	46.60 (3.06)	48.87(2.72)	**0.028**
Block-Tapping	1.86 (0.44)	2.50 (0.50)	**0.001**
Digit Span	1.93(0.37)	2.10 (0.33)	**0.209**
Absolute Gamma (Closed Eyes) After	1.47 (0.69)	3.02 (1.33)	**0.001**
Absolute Gamma (Open Eyes) After	3.15 (2.39)	3.84(2.08)	**0.408**
Relative Gamma (Open Eyes) After	2.75 (1.11)	2.15 (1.45)	**0.21**
Relative Gamma (Closed Eyes) After	4.29(1.44)	2.60 (1.50)	**0.004**

4 DISCUSSION

This study investigated the effects of auditory gamma-band stimulation on cognitive function and frontal gamma oscillatory activity in patients with early-stage Alzheimer's disease (AD). The results demonstrated significant improvements in visuospatial working memory (measured by the Corsi Block-Tapping Task), cognitive flexibility (assessed by the Wisconsin Card Sorting Test), and frontal gamma power during the eyes-closed condition.

These cognitive enhancements align with previous animal studies showing that 40 Hz auditory or visual stimulation can boost microglial activity and reduce amyloid plaque burden, particularly in the prefrontal cortex and hippocampus (Martorell et al., 2019; Iaccarino et al., 2016). Gamma-band oscillations are critically involved in working memory, attention, and sensory integration, and their disruption has been widely documented in both AD patients and transgenic animal models (Verret et al., 2012; Colgin & Moser, 2010). In our study, gamma stimulation sessions were administered in a controlled environment using binaural beats, with strict monitoring of participant adherence and session quality. Notably, the increase in gamma power was observed only in the eyes-closed state, suggesting that frontal resting-state networks may be more responsive to frequency-based entrainment when sensory input is minimized. Importantly, no significant improvements were found in phonological working memory (Digit Span task), which may indicate differential involvement of neural circuits less sensitive to auditory entrainment. This selective effect highlights the importance of task modality and frequency-specific

targeting in cognitive rehabilitation strategies for AD. The selective enhancement of gamma power observed only in the eyes-closed condition may reflect reduced sensory interference and greater engagement of intrinsic neural networks such as the default mode network.

Alzheimer's disease is a complex neurodegenerative disorder with limited treatment options. Gamma-band stimulation, particularly at 40 Hz, has emerged as a promising non-pharmacological approach due to its association with cognitive functions and its ability to modulate disrupted neural oscillations characteristic of AD (Palop et al., 2016). External stimulation in this frequency range elicits predictable neural responses (such as event-related potentials and steady-state responses), which can be harnessed therapeutically. Previous human studies have demonstrated the role of gamma oscillations in visual feature binding, memory, and higher cognitive functions (Tallon-Baudry et al., 1999; Van Wijk et al., 2010; Miller et al., 2018; Griffiths et al., 2019; Benussi et al., 2021; Traikapi & Konstantinou, 2021). Gamma rhythms are notably prominent in the hippocampus, where they contribute to memory processing (Colgin & Moser, 2010; Carr et al., 2012; Buzsáki, 2015). Disruptions in gamma activity have been linked to memory impairments typical of AD, and restoration of gamma oscillations via auditory stimulation may enhance cognitive functions.

Moreover, research by Goutagny et al. (2013) demonstrated that reductions in slow gamma activity (25–50 Hz) in the hippocampal CA1 region contribute to memory deficits. Thus, synchronization at 40 Hz could modulate neural oscillations to improve gamma power and cognitive outcomes. Clinical and preclinical studies support the therapeutic potential of 40 Hz stimulation in reducing amyloid pathology and improving cognition (Iaccarino et al., 2016; Clements-Cortes et al., 2016). While limitations remain, gamma-based auditory stimulation offers a promising, non-invasive approach for AD treatment, potentially serving as an adjunct to pharmacological therapies. Additionally, caregivers reported improvements in mood, motivation, emotional regulation, and reduced depressive symptoms in participants receiving auditory stimulation, warranting further exploration of mood-related benefits.

The apparent discrepancy between increased absolute gamma power and reduced relative gamma power likely reflects normalization effects, whereby increases in total broadband power influence relative spectral proportions. This finding underscores the importance of cautious interpretation of relative power metrics.

Given the limited existing data on gamma-band auditory stimulation in AD, further research with larger samples and diverse populations is necessary. Interdisciplinary collaboration across neurology, cognitive rehabilitation, and related fields will be essential to optimize this therapeutic approach and potentially slow AD progression while improving quality of life. Moreover, it should be acknowledged that part of the intervention group has been described in a previous behavioral study. However, the current manuscript addresses distinct research questions by integrating electrophysiological outcomes and a controlled design, thereby providing novel mechanistic insights into gamma-band auditory stimulation in Alzheimer's disease.

An important methodological limitation of the present study concerns the control stimulus. Although the control condition lacked structured gamma-frequency binaural modulation, broadband auditory stimuli may still contain energy across multiple frequency bands, including gamma. Because detailed spectral analyses (e.g., power spectral density or spectrograms) were not performed, the findings should be interpreted as reflecting the effects

of rhythmic gamma entrainment rather than strictly gamma-specific acoustic exposure. Future studies should incorporate spectrally verified control stimuli and detailed acoustic analyses.

Another limitation of the present study is that EEG recordings were obtained only before and after the intervention and not during auditory stimulation sessions. Therefore, direct measures of neural entrainment, such as steady-state auditory evoked responses or phase-locking indices, could not be assessed. In addition, the spectral analysis focused primarily on the gamma band, and potential effects on other frequency bands (delta, theta, alpha, and beta) cannot be excluded.

A methodological limitation of the present study is that the control stimulus may have contained broadband acoustic energy, including frequencies within the gamma range. Consequently, the observed effects should be interpreted as reflecting the impact of rhythmic gamma-band entrainment rather than exclusive exposure to gamma-band acoustic energy.

Because EEG was not recorded during auditory stimulation, direct indices of neural entrainment, such as auditory steady-state responses or phase-locking measures, could not be assessed. Additionally, spectral analysis was restricted to the gamma band, and potential effects in other frequency bands (delta, theta, alpha, beta) were not evaluated. The electrode montage focused primarily on frontal sites, which may have limited the detection of stimulation-related effects in other cortical regions.

Correlational analyses between changes in gamma power and cognitive improvements were not conducted due to the limited statistical power associated with the small sample size.

The relatively small sample size limits statistical power and increases the risk of both Type I and Type II errors. Accordingly, the findings should be interpreted as preliminary and hypothesis-generating rather than confirmatory.

5 Conclusion

Several limitations of the present study should be acknowledged. First, the relatively small sample size restricted the statistical power and limited the generalizability of the findings. Second, EEG data were not recorded concurrently during the auditory stimulation sessions, thereby precluding direct assessment of phase-locked neural entrainment, such as steady-state evoked responses. Third, due to the perceptible nature of the auditory intervention, participant blinding was not feasible, potentially introducing bias. Finally, mood changes reported anecdotally by caregivers were not measured using standardized psychometric instruments, highlighting the need for more rigorous assessment in future work.

Future research should address these limitations by:

- Recruiting larger, multi-center cohorts to enhance statistical robustness and external validity;
- Incorporating simultaneous EEG recording during stimulation to directly capture steady-state neural responses and entrainment dynamics;
- Implementing longitudinal study designs to evaluate the persistence and durability of cognitive improvements over time;
- Exploring multi-sensory entrainment protocols, such as combined visual and auditory gamma stimulation, to potentially augment therapeutic efficacy.

Auditory stimulation within the gamma frequency band (40 Hz) represents a promising non-pharmacological intervention for improving cognitive functions in individuals with early-stage Alzheimer's disease. Our results demonstrate that repeated exposure to gamma-band auditory beats can enhance visuospatial working memory and cognitive flexibility, accompanied by increased frontal gamma oscillatory activity, particularly in the eyes-closed resting state.

The domain-specific effects observed suggest that auditory entrainment may selectively modulate neural networks implicated in executive functioning and spatial cognition. Given its non-invasive, low-cost, and easily implementable nature, auditory gamma stimulation holds potential as a complementary therapy alongside conventional pharmacological treatments. Nevertheless, further investigations involving larger samples, extended follow-up periods, and advanced neurophysiological monitoring are required to validate the robustness and long-term sustainability of these cognitive benefits, as well as to elucidate the underlying neural mechanisms in greater depth.

Acknowledgment

This study was supported by the Cognitive Science and Technologies Council. The authors extend their gratitude to the participants and their caregivers for their cooperation.

The authors would like to express their heartfelt gratitude to Dr. Mahdi Farhoudi, a Neurology Professor at Tabriz University of Medical Sciences, for his invaluable assistance in identifying, screening, and recruiting Alzheimer's patients for this study. His dedication and expertise were instrumental in ensuring the success of this research.

Author Contributions

Leila Mehdizadeh Fanid: Supervising the work, Being responsible for all data, figures, and text, Ensuring that authorship is granted appropriately to contributors, Identifying and declaring conflicts of interest on behalf of all authors, Identifying and disclosing related work by any co-authors under consideration elsewhere, Formal analysis, Supervision, Investigation, Project administration.

Mansour Beyrami: Methodology**.**

Mahdi Jafari Asl: Ensuring that authorship is granted appropriately to contributors, Ensuring adherence to all editorial and submission policies, Identifying and declaring conflicts of interest on behalf of all authors, Arbitrating decisions and disputes and ensuring communication with the journal (before and after publication), sharing of any relevant information or updates to co-authors, and being accountable for fulfilment of requests for reagents and resources, Writing – review & editing**.**

Behzad Nikzad: Resources**.**

Siamak Dadashi: Methodology, Data curation, Investigation**.**

Zahra Zehtabi: Writing – original draft, Formal analysis, Supervision**.**

Habibollah Rasouli: Methodology, Data curation.

Declaration of interests

The authors declare no competing interests.

REFERENCES

Benussi A., Cantoni V., Cotelli M. S., Cotelli M., Brattini C., Datta A., et al. (2021). Exposure to gamma tACS in Alzheimer's disease: a randomized, double-blind, sham-controlled, crossover, pilot study. *Brain Stimul.* 14 531–540. 10.1016/j.brs.2021.03.007

Bosman CA, Lansink CS, and Pennartz CM. Functions of gamma-band synchronization in cognition: from single circuits to functional diversity across cortical and subcortical systems. Eur J Neurosci. 2014 Jun; 39(11):1982-99. Doi: 10.1111/ejn.12606. Epub 2014 May 8. PMID: 24809619.

Buzsáki G. (2015). Hippocampal sharp wave-ripple: a cognitive biomarker for episodic memory and planning. *Hippocampus* 25 1073–1188. 10.1002/hipo.22488

Cantor, D. S., & Evans, J. R. (Eds.). (2013). *Clinical neurotherapy: application of techniques for treatment*. Academic Press.

Carr M. F., Karlsson M. P., Frank L. M. (2012). Transient slow gamma synchrony underlies hippocampal memory replay. *Neuron* 75 700–713. 10.1016/j.neuron.2012.06.014

Chaieb, L., Wilpert, E. C., Reber, T. P., & Fell, J. (2015). Auditory beat stimulation and its effects on cognition and mood states. Frontiers in psychiatry, 6, 70.

Chaudhury, S., Nag, T. C., Jain, S., & Wadhwa, S. (2013). Role of sound stimulation in reprogramming brain connectivity. *Journal of Biosciences*, *38*(3), 605-614.

Clements-Cortes, A., Ahonen, H., Evans, M., Freedman, M., & Bartel, L. (2016). Short-term effects of rhythmic sensory stimulation in Alzheimer's disease: An exploratory pilot study. *Journal of Alzheimer's Disease*, *52*(2), 651-660.

Colgin, L. L. (2016). Rhythms of the hippocampal network. *Nature Reviews Neuroscience*, *17*(4), 239-249.

Colgin, L. L., & Moser, E. I. (2010). Gamma oscillations in the hippocampus. *Physiology*, *25*(5), 319-329.

Corsi, P.M. (1972). Human memory and the medial temporal region of the brain (Ph.D.). McGill University.

Fukui, H., & Toyoshima, K. (2008). Music facilitates the neurogenesis, regeneration, and repair of neurons. *Medical hypotheses*, *71*(5), 765-769.

Galambos, R., Makeig, S., & Talmachoff, P. J. (1981). A 40-Hz auditory potential recorded from the human scalp. Proceedings of the national academy of sciences, 78(4), 2643-2647.

Gillespie, A. K., Jones, E. A., Lin, Y. H., Karlsson, M. P., Kay, K., Yoon, S. Y., ... & Huang, Y. (2016). Apolipoprotein E4 causes age-dependent disruption of slow gamma oscillations during hippocampal sharp-wave ripples. *Neuron*, *90*(4), 740-751.

Goutagny R., Gu N., Cavanagh C., Jackson J., Chabot J. G., Quirion R., et al. (2013). Alterations in hippocampal network oscillations and theta-gamma coupling arise before Aβ overproduction in a mouse model of Alzheimer's disease. *Eur. J. Neurosci.* 37 1896–1902. 10.1111/ejn.12233

Grant, D. A., & Berg, E. A. (1948). A behavioral analysis of the degree of impairment and ease of shifting to new responses in a Weigl-type card sorting problem. *Journal of Experimental Psychology*, *39*, 404–411.

Griffiths B. J., Parish G., Roux F., Michelmann S., Plas M., van der, et al. (2019). Directional coupling of slow and fast hippocampal gamma with neocortical alpha/beta oscillations in human episodic memory. *Proc. Natl. Acad. Sci. U S A.* 116 21834–21842. 10.1073/PNAS.1914180116

Herrmann, C. S., Munk, M. H., & Engel, A. K. (2004). Cognitive functions of gamma-band activity: memory match and utilization. *Trends in cognitive sciences*, *8*(8), 347-355.

Herrmann, C. Human EEG responses to 1–100 Hz flicker: resonance phenomena in visual cortex and their potential correlation to cognitive phenomena. Exp Brain Res 137, 346–353 (2001). https://doi.org/10.1007/s002210100682

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., & Adaikkan, C. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature*, *540*(7632), 230-235.

Kessels, R. P. C., van Zandvoort, M. J. E., Postma, A., Kappelle, L. J., de Haan, E. H. F. (2000). The Corsi Block-Tapping Task: Standardization and Normative Data. *Applied Neuropsychology 7*, 252–258.

Kumar, A., & Singh, A. (2015). A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacological reports*, *67*(2), 195-203.

Lambon Ralph, M. A., Patterson, K., Graham, N., Dawson, K., & Hodges, J. R. (2003). Homogeneity and heterogeneity in mild cognitive impairment and Alzheimer's disease: a cross-sectional and longitudinal study of 55 cases. *Brain*, *126*(11), 2350-2362.

Lezak, M. D. (2004). *Neuropsychological assessment*. Oxford University Press, USA.

Mably, A. J., Gereke, B. J., Jones, D. T., & Colgin, L. L. (2017). Impairments in spatial representations and rhythmic coordination of place cells in the 3xTg mouse model of Alzheimer's disease. *Hippocampus*, *27*(4), 378-392.

Martorell, A. J., Paulson, A. L., Suk, H. J., Abdurrob, F., Drummond, G. T., Guan, W., & Tsai, L. H. (2019). Multi-sensory gamma stimulation ameliorates Alzheimer ' 's-associated pathology and improves cognition. *Cell*, *177*(2), 256-271.

McDermott, B., Porter, E., Hughes, D., McGinley, B., Lang, M., O'Halloran, M., & Jones, M. (2018). Gamma band neural stimulation in humans and the promise of a new modality to prevent and treat Alzheimer's disease. *Journal of Alzheimer's Disease*, *65*(2), 363-392.

McLeod, S. (2008). Working Memory, Simply Psychology, viewed 21 September 2015, http://www.simplypsychology.org/working%20memory.

Mehdizadeh Fanid, L., Birami, M., Nikzad, B., Dadashi, S., Rasouli, H., & Zarezadeh, N. (2025). The Effect of Auditory Brain Stimulation on Cognitive Function Improvement: A Study on Working Memory and Cognitive Flexibility in Adults With Early-Stage Alzheimer's. The Scientific Journal of Rehabilitation Medicine, 14(5), 762-773. doi: 10.32598/SJRM.14.5.3370

Miller E. K., Lundqvist M., Bastos A. M. (2018). Working memory 2.0. *Neuron* 100 463–475.

Moraes, M. M., Rabelo, P. C., Pinto, V. A., Pires, W., Wanner, S. P., Szawka, R. E., & Soares, D. D. (2018). Auditory stimulation by exposure to melodic music increases dopamine and serotonin activities in rat forebrain areas linked to reward and motor control. *Neuroscience letters*, *673*, 73-78.

Nakazawa, K., Quirk, M. C., Chitwood, R. A., Watanabe, M., Yeckel, M. F., Sun, L. D., & Tonegawa, S. (2002). Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science*, *297*(5579), 211-218.

Palop, J. J., & Mucke, L. (2016). Network abnormalities and interneuron dysfunction in Alzheimer's disease. *Nature Reviews Neuroscience*, *17*(12), 777-792.

Piper, B. J., Mueller, S. T., Geerken, A. R., Dixon, K. L., Kroliczak, G., Olsen, R. H., & Miller, J. K. (2015). Reliability and validity of neurobehavioral function on the Psychology Experimental Building Language test battery in young adults. *PeerJ*, *3*, e1460.

Reedijk, S. A., Bolders, A., & Hommel, B. (2013). The impact of binaural beats on creativity. Frontiers in human neuroscience, 7, 69795.

Ross, B., Jamali, S., & Tremblay, K. L. (2013). Plasticity in neuromagnetic cortical responses suggests enhanced auditory object representation. BMC neuroscience, 14(1), 151.

Roy, D. S., Arons, A., Mitchell, T. I., Pignatelli, M., Ryan, T. J., & Tonegawa, S. (2016). Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature*, *531*(7595), 508-512.

Scheltens, P., Blennow, K., Breteler, M. M., De Strooper, B., Frisoni, G. B., & Salloway, S. (2016). Van der Flier WM: Alzheimer's disease. *Lancet*, *388*(10043), 505-17.

Stopford, C. L., Thompson, J. C., Neary, D., Richardson, A. M., & Snowden, J. S. (2012). Working memory, attention, and executive function in Alzheimer's disease and frontotemporal dementia. *Cortex*, *48*(4), 429-446.

Tallon-Baudry C, Bertrand O. Oscillatory gamma activity in humans and its role in object representation. *Trends Cogn Sci*. 1999 Apr; 3 (4):151-162. Doi: 10.1016/s1364-6613(99)01299-1. PMID: 10322469.

Thut, G., Schyns, P., & Gross, J. (2011). Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. *Frontiers in Psychology*, *2*, 170.

Toepper, M., Gebhardt, H., Beblo, T., Thomas, C., Driessen, M., Bischoff, M., & Sammer, G. (2010). Functional correlates of distractor suppression during spatial working memory encoding. *Neuroscience*, *165*(4), 1244-1253.

Traikapi, A., & Konstantinou, N. (2021). Gamma Oscillations in Alzheimer's disease and Their Potential Therapeutic Role. Frontiers in Systems Neuroscience, 15, 782399. https://doi.org/10.3389/fnsys.2021.782399

Van Wijk, B. C. M., Stam, C. J., & Daffertshofer, A. (2010). Comparing brain networks of different sizes and connectivity density using graph theory. *PLoS ONE*, *5*(10), e13701. https://doi.org/10.1371/journal.pone.0013701

Vandierendonck, A., Kemps, E., Fastame, M. C., & Szmalec, A. (2004). Working memory components of the Corsi blocks task. *British Journal of Psychology*, *95*(1), 57-79.

Verret, L., Mann, E. O., Hang, G. B., Barth, A. M., Cobos, I., Ho, K., & Mucke, L. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in the Alzheimer model. *Cell*, *149*(3), 708-721.

Walker, S. P., Chang, S. M., Younger, N., & GRANTHAM-MCGREGOR, S. M. (2010). The effect of psychosocial stimulation on cognition and behavior at 6 years in a cohort of term, low-birth-weight Jamaican children. *Developmental Medicine & Child Neurology*, *52*(7), e148-e154.