Investigating the Impact of Theta tACS on Resting State Networks in patients with Amnestic Mild Cognitive Impairment: A Graph Theory Study

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Abstract

Alzheimer's disease (AD) presents a significant economic and social challenge, with the number of patients expected to rise in the coming decades. Mild cognitive impairment (MCI) is a transitional stage between healthy cognitive aging and AD and has been identified as a critical window of opportunity to improve or retain cognitive functioning. Theta transcranial alternative current stimulation (tACS) has the potential to enhance cognitive functioning by improving brain network synchronization. However, the effects of tACS on disrupted brain network functioning in individuals with aMCI are largely unknown. To close this gap, the current study investigated the effects of theta tACS on resting state functional connectivity (rs-FC) in individuals with aMCI using functional magnetic resonance imaging (fMRI). The focus was on two networks involved in cognition that are affected by the pathophysiological disruptions associated with the dementing process, namely the Default Mode Network (DMN) and Frontoparietal Network (FPN). Patients with aMCI (N=47) were randomly allocated to one of three conditions: 6Hz theta tACS (n=12), individualized theta tACS (n=18), or sham tACS (n=17). Following ten consecutive days of fifteen-minute stimulation, pre- and post-measurements of rs-FC graph theory metrics were compared. Results revealed no statistically significant differences in DMN and FPN rs-FC following theta tACS, indicating that the parameters tested in this study did not modulate functional connectivity in patients with aMCI. Alternative interpretations and limitations of the study as well as recommendations for future research are discussed. The results highlight the need to refine stimulation parameters and rs-FC analysis approaches to further investigate the neural underpinnings of tACS effects in the aMCI population.

Keywords: Theta tACS, resting-state functional connectivity, aMCI, graph theory, Devault Mode Network, Frontoparietal Network

Theoretical Background

The number of patients with Alzheimer’s disease (AD) within the European Union is expected to double by 2040, from 7.5 million patients in 2013 to 13.1 million patients (Tomaskova et al., 2016). This is particularly concerning given the significant economic burden of dementia in Europe which with amounted to 177 billion euros in 2008 alone, and the profound consequences of severe cognitive decline on daily living and quality of life (Lee et al., 2018; Wimo et al., 2011). Mild cognitive impairment (MCI) is considered a transitional stage on the continuum between healthy cognitive aging and AD, in which individuals experience cognitive impairment to some degree but can still function independently. Researchers have identified MCI as a critical period for disease altering intervention. Changing the disease course at this stage by slowing the cognitive decline that occurs during the progression from MCI to AD would have enormous economic and social value (Alzheimer’s Association, 2015). Yet, most attempts to consistently improve cognition in individuals with MCI have failed. Non-invasive brain stimulation (NIBS) techniques have gained increasing attention over recent decades as methods to understand and enhance brain functioning in individuals with neurological disorders (Cantone et al., 2021). One of these techniques, transcranial alternating current stimulation (tACS), is a promising candidate for improving cognitive functioning in individuals with MCI. However, the neural mechanisms underlying its treatment effects in this population remain largely understudied. To close this gap, the current study aimed to investigate the effects of theta tACS on resting state functional connectivity in individuals with amnestic MCI (aMCI) using functional magnetic resonance imaging (fMRI). The primary objective is to understand the influence of tACS on the organization and functioning of large-scale brain networks involved in cognition, namely the Default Mode Network (DMN) and Fronto-Parietal Network (FPN). These networks are increasingly disconnected over the neurodegenerative processes of MCI and AD which is suggested to contribute to cognitive dysfunction (Delbeuck et al., 2003; Eyler et al., 2019; Li et al., 2016; Phillips et al., 2015; Yang et al., 2023). A comprehensive understanding of the neural mechanism underlying tACS treatment effects in individuals with aMCI could contribute to the identification of optimal stimulation parameters and early markers of treatment efficacy. This requires investigating how brain networks affected by disconnection due to cerebral atrophy respond to stimulation (Tabatabaei-Jafari et al., 2015).

Mild Cognitive Impairment: Symptoms and Treatment

MCI is defined as a cognitive decline greater than expected for an individual's age and level of education without interfering notably with activities of daily living (Gauthier et al., 2006). It is distinct from dementia, in which cognitive deficits hinder independent functioning. However, individuals with MCI face a significantly higher risk of developing AD or other dementias. Patients with the amnestic subtype (aMCI) and concurrent executive functioning deficits are especially likely to develop AD (Kramer et al., 2006; Rozzini et al., 2007). Estimated annual conversion rates from aMCI to AD are between 10-18% and up to 80% of individuals with aMCI develop AD within 6 years following diagnosis (Petersen, 2004, Gauthier et al., 2006,). Of note, a minority of patients diagnosed with MCI will not develop dementia in the subsequent years and their cognition can even improve towards normal levels.

Clinical drug trials for AD have failed to deliver an effective treatment in the past decade, potentially because AD brain pathology starts to develop in the preclinical stages of AD and may be too severe by the time of AD diagnosis (Cummings et al., 2014; Sperling et al., 2013). This has led researchers to target cognitive and brain functioning during the early MCI stage in an effort to delay progression towards AD. Economic analyses suggest that disease altering treatment in the preclinical phase of dementia could significantly reduce health care related costs (Alzheimer's Association, 2015). Yet, to this date, there are no effective clinical treatments to slow the deterioration of cognitive functioning and progression of brain pathology in individuals with aMCI (Cooper et al., 2013). Pharmacological interventions with anticholinergic drugs remain the most common approach, despite only limited evidence for success in improving cognitive functioning. Efforts to improve cognitive outcomes with computerized cognitive training have given reason for hope, however the lack of long term data and variation in methodologies render these findings hard to interpret (Sherman et al., 2017; Zhang et al., 2019).

Given the challenge of aging populations in most western countries and the consequently rising number of individuals with (a)MCI and AD, there is a pressing need to develop effective disease altering treatments to reduce costs and burden for the healthcare system, the affected individuals and their relatives (Jones et al., 2023).

Transcranial Alternating Current Stimulation

Transcranial Alternating Current Stimulation is a promising non-invasive brain stimulation technique that could deliver such treatment by modulating cortical excitability and neuroplasticity to improve cognitive functioning in individuals with aMCI (Nissim et al., 2023). This technique modulates neural activity through the application of weak sinusoidal oscillating electric currents to the scalp (Klink et al., 2020). Endogenous frequencies generated in the brain represent a neural mechanism that drives various cognitive functions (Sejnowski & Paulsen, 2006). Through a mechanism called Entrainment, tACS targets these endogenous cortical oscillations in a frequency-dependent manner to regulate and synchronize network communication (Elyamany et al., 2021; Nissim et al., 2023). Intrinsic oscillations temporally synchronize to the externally applied electric field which is proposed to account for the impact of tACS on cognitive performance when applied during a task (online effects) (Klink et al., 2020). Moreover, the effects of tACS can outlast the stimulation period itself (offline effects) which illustrates its potential as a therapeutic intervention (Veniero et al., 2015). These long-term effects on cognitive performance are thought to reflect plasticity-related network changes as a consequence of repeated periods of entrainment (Bland & Sale, 2019).

The ability of tACS to influence endogenous oscillations in a frequency-dependent manner allows it to selectively target cognitive functions that are associated with certain frequency bands (Elyamany et al., 2021; Sejnowski & Paulsen, 2006). For interventions designed to improve cognition (or delay decline) in aMCI, researchers have focused on frequencies associated with episodic memory and cognitive control (i.e. working memory and executive functioning).

For example, hippocampal-mediated memory processes, which are disrupted in the early disease stages of AD and aMCI, are driven by oscillations in the gamma frequency range (30-80 Hz) (Griffiths et al., 2019; Güntekin et al., 2023). The application of tACS at a gamma frequency has shown promising results in improving memory and cognition in individuals with aMCI (Nissim et al., 2023).

tACS in the theta frequency range (4-7 Hz) presents another approach to intervention in aMCI. Synchronized oscillations in the theta range are associated with executive functioning (EF) and working memory (WM) (Hsieh & Ranganath, 2014; Sauseng et al., 2010). EF and WM deficits increase the likelihood of progressing from aMCI to AD, beyond the impact of memory impairment alone (Rozzini et al., 2007). Consequently, by improving these functions with tACS, the disease trajectory may be altered and conversion to AD delayed. A recent meta-analysis, reviewing 30 studies, found significant positive online and offline effects of theta tACS on executive functioning and working memory in young healthy adults (Lee et al., 2023). In another study, prefrontal theta tACS at 6 Hz combined with cognitive training significantly improved attention and inhibitory control functions in patients with aMCI, while cognitive control training combined with sham tACS was ineffective (Jones et al., 2023). The ability of tACS to improve WM and EF is promising given their central role in the aMCI disease trajectory. However, it remains to be investigated whether theta tACS can affect long term cognitive functioning and conversion rates from aMCI to AD.

Another parameter to consider for tACS treatment is whether stimulation should be applied at an individual or at a standardized frequency across all patients (Nissim et al., 2023). According to the theory of entrainment, intrinsic brain rhythms will only be synchronized if the externally applied field oscillates at the same frequency. Applying tACS at individualized peak frequencies, which are determined by electroencephalogram (EEG) measurement prior to stimulation, might therefore have a more effective impact on brain synchronization and network communication.

As mentioned above, the long-term offline effects of tACS on cognitive functioning are believed to stem from plasticity-related changes in large-scale functional networks (Bland & Sale, 2019). Studies utilizing several neuroimaging methods, including EEG and functional magnetic resonance imaging (fMRI), have provided convincing evidence that tACS exerts downstream influences on connectivity and coherence in brain networks associated with cognition (Khan et al., 2023; Neuling et al., 2013). However, research on tACS treatment for patients with aMCI has primarily focused on cognitive and behavioral outcomes. A comprehensive investigation of the long-term effects of stimulation on functional networks known to be disrupted as a consequence of brain pathology associated with aMCI and AD remains to be undertaken.

Resting-State Functional Connectivity in MCI

Resting state fMRI (rs-fMRI) is a powerful tool to explore network functioning in individuals with aMCI. Rs-fMRI non-invasively measures inherent activity fluctuations from the whole brain and allows insights into the brain’s functional organization (Bijsterbosch et al., 2020). Resting state functional connectivity (rs-FC) refers to temporal correlations in the spontaneous fluctuations of the blood oxygenation level dependent (BOLD) signal. It can be used to investigate functional relationships across spatially distinct brain areas. Functional connectivity studies have identified several brain networks involved in complex behavior including the Default Mode Network (DMN), Dorsal Attention Network (DAN), and Fronto-Parietal Network (FPN) (Raichle, 2015; Zanto & Gazzaley, 2013). The DMN and FPN are interacting networks that are especially relevant for the study of aMCI, given their roles in memory functioning and cognitive control. The DMN is involved in episodic and autobiographical memory processing and shows decreased activity during task-oriented cognitive processing (Raichle, 2015). The FPN is a critical hub for cognitive control which becomes active when task-oriented cognitive processing is demanded (Marek & Dosenbach, 2018). These networks can be studied across different disease states or before and after therapeutic interventions to investigate the neural underpinnings of disease and treatment (Bijsterbosch et al., 2017).

Structural and functional evidence points towards AD as a type of “disconnection syndrome” (Delbeuck et al., 2003; Liang et al., 2011). Disrupted functional connectivity between structures of the DMN and FPN are observed already in the early stages of AD and aMCI (Eyler et al., 2019; Yang et al., 2023). Once patients progress towards AD, there are consistent differences in resting state networks, especially the DMN, reflected by reduced functional connectivity in patients compared to healthy controls (Binnewijzend et al., 2012). This altered functional connectivity within the DMN correlates with memory performance and cognitive functioning indicating a role of functional disconnection in the cognitive decline associated with aMCI (Gardini et al., 2015; Liang et al., 2011). Both increased and decreased connectivity within the DMN of aMCI patients compared to healthy controls have been observed (Eyler et al., 2019). This ambivalent pattern of functional connectivity alterations was interpreted as reflecting both mechanism and compensation for cognitive deficits (Wang et al., 2013; Wiepert et al., 2017). That is, reduced connectivity in posterior parts of the DMN, especially the posterior cingulate cortex, could result from neuropathological changes associated with AD and contribute to cognitive dysfunction. Increased functional connectivity in prefrontal regions, on the other hand, could reflect compensatory mechanisms for the disruptions in more posterior regions (Gardini et al., 2015; Liang et al., 2011). A recent meta-analysis demonstrated altered FPN resting state functional connectivity in patients with MCI (Yang et al., 2023). This could represent part of the pathophysiological mechanism underlying executive functioning deficits in aMCI.

In sum, disruptions in neural networks are characteristic of aMCI and AD and are likely to contribute to cognitive dysfunction. It is possible that tACS could be used as an intervention to affect these disrupted functional networks in order to improve cognition. However, this hypothesis has not been tested, and it remains unclear whether tACS affects resting state networks in individuals with aMCI.

Investigating the Effects of theta tACS on DMN and FPN Functional Connectivity

The aim of this paper is to bridge a gap in the current literature by examining the impact of theta tACS on functional connectivity within key resting-state networks in individuals with aMCI, namely the DMN and FPN. Given the disruptions in network functioning and brain atrophy associated with aMCI and AD, this study investigated whether tACS, through its capacity to synchronize brain oscillations, can alter activity within these increasingly disconnected networks (Tabatabaei-Jafari et al., 2015). Additionally, the role of individualizing the stimulation frequencies was addressed by comparing the effects of tACS delivered at a standardized versus an individualized theta frequency.

A graph theory approach was chosen to analyze functional connectivity data. Graph theory is a branch of mathematics that describes properties of complex networks by reducing them to an assortment of nodes and edges (Van Diessen et al., 2014). This mathematical framework can be applied to brain network analysis, where brain regions are represented as nodes and the connections between them (i.e. the correlation in resting state activity) as edges. From these two elements, mathematical inferences can be drawn to characterize properties of brain networks. Neural networks are organized according to a “small world” architecture, meaning that anatomically close brain regions have more connections than distant regions (Bullmore & Sporns, 2009; Fair et al., 2009; Openneer et al., 2020). This “small world” architecture, which is fundamental to efficient information processing, is altered in individuals with MCI (Phillips et al., 2015). In this study, two graph metrics reflecting “small-world” measures of functional connectivity – clustering coefficient and characteristic path length - were chosen to quantify the effect of tACS on resting state networks in MCI patients (see methods section for graph metrics).

In this study, rs-FC in the DMN and FPN was compared pre- and post-stimulation across three groups. Two groups received active tACS to the frontal and parietal lobes, either at an individualized or standardized (6Hz) theta frequency, while simultaneously performing a cognitive task. In the control group, aMCI patients received sham tACS while simultaneously performing the same cognitive task. It was hypothesized that both active forms of tACS would show a stronger influence on the graph metrics of rs-FC within the FPN and DMN compared to sham stimulation. In line with the principles of entrainment, stimulation at an individualized theta frequency was expected to show stronger effects on network connectivity than tACS delivered at a standardized 6Hz theta frequency.

Methods

The data presented in this thesis is derived from the larger “Cogmax” study (coordinated by Dr. Branislava Curcic Blake) which was approved by the Medical Ethical Committee of the University Medical Center Groningen (UMCG). The study was conducted over several months to investigate the impact of tACS on cognitive and brain functioning in individuals with aMCI. For this paper, the scope of the methods section will be narrowed to outline methodology and procedures relevant to examine rs-FC changes induced by tACS.

Study Design

This study adopted a between subjects, randomized, double-blind, and sham controlled design. After being screened for inclusion and exclusion criteria and results of the neuropsychological evaluation (see below), participants were randomly assigned to one of three groups, i.e., sham stimulation, standardized theta stimulation, and individualized theta stimulation. The experiment had a total duration of 11 days. On day one, subjects were asked to fill out demographic information (i.e. level of education and handedness) and to undergo baseline resting-state fMRI. Next, patients received either active or sham tACS 10 times on 10 consecutive days. During the 15-minute stimulation sessions, patients were asked to perform two working memory paradigms - the two-letter delayed and the digit span forward and backward tasks (Polanía et al., 2012; Vosskuhl et al., 2015). Participants engaged in the tasks because performing a task while receiving stimulation can enhance the effects of the stimulation (Paulus, 2011). After the stimulation procedure was complete on day 11, patients underwent post-stimulation resting state fMRI. Blinding efficacy was assessed by asking participants to estimate whether they were in the real or sham condition before finally, the experimental condition was revealed.

Participants

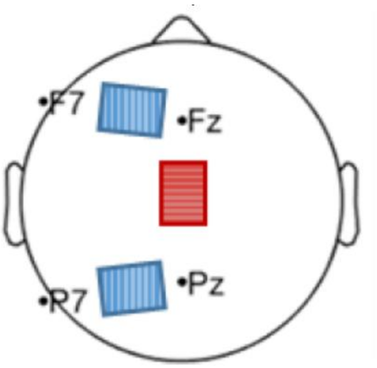
In total, 56 patients with a diagnosis of aMCI aged 50 years or older were recruited from the memory clinic of the UMCG and general practitioners in the Groningen area. Additionally, the study was advertised via media and patient societies. A neuropsychological evaluation confirmed the aMCI diagnosis, identifying those patients with a verbal memory score of 1.5 standard deviations below the normative comparison standard on the Rey Auditory Verbal Learning Test. The exclusion criteria consisted of 1.) history of psychiatric or neurological illness other than MCI, 2.) metal implants, 3.) risk of having metal in the eyes, 4.) tattoos containing iron oxide, 5.) pregnancy or breastfeeding, 6.) claustrophobia, 7.) alcohol or drug abuse, 8.) recent alcohol use, 9.) refusal to be informed of structural brain abnormalities identified during MRI, 10.) severe scalp skin lesions, and 11.) color blindness. Nine participants had to be excluded from the analysis because of missing imaging data (n=8) or bad image quality (n=1) resulting in a final study sample of N=47. The groups were comprised of n=17 subjects in the sham condition, n=12 subjects in the standardized theta stimulation condition, and n=18 participants in the individualized theta stimulation condition. Demographic characteristics are summarized in Table 2 below.

Stimulation Parameters

Patients were fitted with two positively charged electrodes (anode) on the left frontal and parietal lobe – at the F3 and P3 positions of the 10/20 EEG system – and a negatively charged cathode at the Cz position (see Figure 1). Each rubber electrode measured 7 x 3 x 1 cm in dimension. The two active groups received tACS delivered at 1mA peak-to-peak amplitude either at an individual relevant theta frequency as performed by Vosskuhl et al., (2015) or at 6Hz (protocol by Polonia, Nitsche et al., 2012). The overall stimulation period lasted for 15 minutes.

Figure 1

Electrode Positioning and Orientation on the Scalp



Note.

Resting-State fMRI

MRI Acquisition

All patients were scanned with a 3 Tesla Siemens Magnetom Prisma MRI scanner equipped with a 32-channel head-coil at the UMCG neuroimaging center. The participants were asked to keep their eyes closed and lie still in the scanner. Anatomical images were acquired using a T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR = 2300 ms; TE = 2.98 ms; Field of View = 256 x 256 x 256 mm3; flip angle = 9 degrees; voxel size = 1 x 1 x 1 mm3; number of slices = 176; acquisition time = 9:14 min). The T2-weighted functional images during rest were acquired with a Multi-Echo Planar imaging sequence (TR = 2170 ms; TE1 = 9.74 ms; TE2 = 22.10 ms; TE3 = 34.46 ms; flip angle = 60 degrees; voxel size = 3 x 3 x 3 mm3; number of slices = 39; number of volumes = 220, acquisition time = 8:28 min)

Preprocessing of functional MRI images

The functional MRI images were initially preprocessed using fMRIprep. Head motion parameters were estimated to correct for head movements and minimize artifacts in the time series data. Slice timing correction was applied to adjust for the temporal offset between the acquisition of different slices. The data was resampled into the native space for motion correction. The original dimensions were preserved. A non-linear regression approach was used to combine the multi-echo sequences, enhancing the signal-to-noise ratio. The functional images were co-registered to the anatomical T1 weighted images with a six-degree of freedom transformation. Finally, to enable comparisons across subjects, images were normalized into the standard Montreal Neurological Institute (MNI) space.

Following the initial preprocessing, the data was further refined using XCP-D during post-processing (Mehta et al., 2023). Automatic head radius estimation was applied by calculating the framewise displacement (FD) to identify motion spikes and despiking was performed. The data was denoised using 24 nuisance regressors to eliminate noise components. A fourth order bandpass filter (0.01Hz to 0.1 Hz) was applied to reduce the signal to frequency range associated with resting-state activity. Where the framewise displacement threshold exceeded 0.5 mm, censoring was used to exclude these timepoints, minimizing the impact of head movements. Finally, spatial smoothing was applied using a Gaussian Kernel of 6mm full width at half maximum (FWHM) to enhance the signal-to-noise ratio.

Region of Interest Definitions and Graph Construction

To perform network statistics, the CONN toolbox implemented in Matlab 2023b was used (Nieto-Castanon, 2020). The toolbox provides resting state network (RS-Network) templates with pre-defined regions of interests (ROI). The core DMN and FPN seed regions are summarized in Table 1. For each ROI, the extracted time series was averaged, and ROI-to-ROI correlation (RRC) matrices were computed based on the average time series to characterize the strengths of functional connectivity between the ROI (For details about RRC measures in CONN see Nieto-Castanon, (2020)). The RRC matrices served as input to perform graph theory analyses.

Table 1

Seed Regions and Coordinates of DMN and FPN provided by RS-Network template in CONN

RS-Network	Region	Coordinates (x, y, z)	Abbreviation
FPN	Left-lateral prefrontal cortex	[-43 +33 +28]	L-LPFC
	Left-posterior prefrontal cortex	[-46 -58 +49]	L-PCC
	Right-posterior parietal cortex	[+41 +38 +30]	R-LPFC
	Right-posterior prefrontal cortex	[+ 52 – 52 +45]	R-PCC
DMN	Medial prefrontal cortex	[+1 +55 -3]	MPFC
	Left-lateral parietal cortex	[-39 -77 +33]	L-LP
	Right-lateral parietal cortex	[+47 -67 +29]	R-LÜ
	Posterior cingulate cortex	[+1 -61 +38]	PPC

Note. Table is adopted from Khan et al. (2023). Coordinates are in MNI space.

Graph Metrics

The average path length and clustering coefficient are two graph metrics commonly used to characterize network organization and small-world architecture (Van Diessen et al., 2014). The average path length is defined as the average shortest-path distance between a node and all other nodes (Achard & Bullmore, 2007; Latora & Marchiori, 2001). It is inversely related to the level of network integration, meaning that a small average path length reflects a high level of integration (Van Diessen et al., 2014). The clustering coefficient is defined as the proportion of connected nodes across all neighboring nodes (Achard & Bullmore, 2007; Latora & Marchiori, 2001). The clustering coefficient represents a measure of network segregation, with a high coefficient indicating a highly segregated network (Van

Diessen et al., 2014). An efficient small word architecture in the brain is associated with a high clustering coefficient and a low average path length (Bullmore & Sporns, 2009).

Statistical Analyses

Group differences in graph metrics (i.e. average path length and clustering coefficient) per network were investigated using the CONN toolbox implemented in Matlab 2023b. The CONN 2nd-level analysis module applies a General Linear Model (GLM) for all second-level analyses (group-comparisons) of functional connectivity data (Nieto-Castanon, 2020). A GLM defines a multivariate linear association between a set of independent variables/measures X and a set of dependent variables/measures Y (Nieto-Castanon, 2020). For functional connectivity analysis, the dependent variable y(n) takes the form of a row vector that encodes functional connectivity values (i.e. Graph metrics) from the n-th subject in the study across one or multiple conditions (i.e. pre-stimulation, post-stimulation). The independent variable x(n) is a row vector that encodes one or several group variables (i.e. Stimulation type) for the same subject.

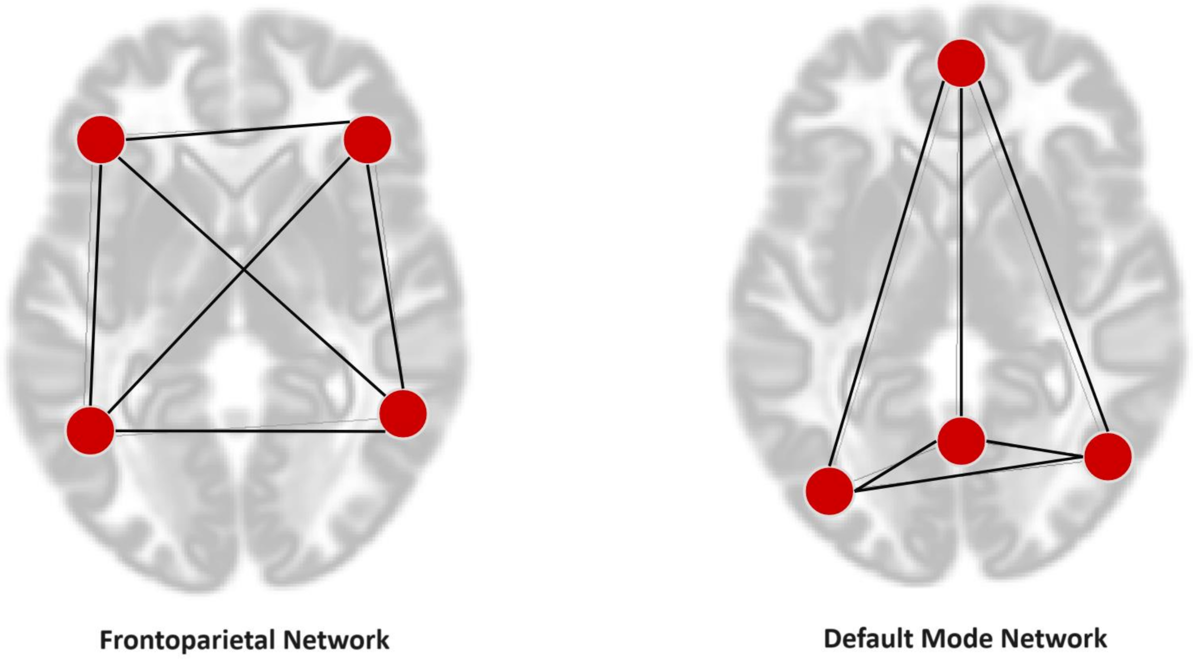
General Linear Model

$$Y(n) = x(n)B+\varepsilon(n)$$

Within the GLM framework, it is possible to perform a large array of classical statistical analyses. Several 3x2 mixed ANOVAs were performed per graph-metric (dependent variable) and network, testing for an interaction effect with stimulation type as between-subjects factor (i.e. 6Hz, individual frequency, sham) and time as within-subjects factor (i.e. pre-stimulation, post-stimulation). An interaction would indicate a differential effect of stimulation type on the graph metrics. The analyses were conducted for the combined networks and at each node individually (see Figure 2). “Combined network” refers to the average of the graph metrics derived from all nodes within a network. This amounted to a total of 20 ANOVAs: four testing for interactions within the combined networks, two for each network, and 16 ANOVAs tested for an interaction between time of measurement and stimulation condition at the individual nodes. To test for direction of effects, post-hoc paired sample t-tests were conducted at the network level**.** The False Discovery Rate (FDR) correction was applied to control for Type I errors due to multiple comparisons. Statistical significance was reached at an FDR-controlled p-value of less than 0.05.

Figure 2

Graphical representation of nodes and edges of the DMN and FPN graphs used in CONN toolbox



Note. Analyses were conducted on the graph metrics at each node of a network individually, and for the average graph metrics across a network. With four nodes in each network and two graph metrics, this resulted in a total of 20 ANOVA's. Brain images were obtained from the CONN user interface and edited (Nieto-Castanon, 2020).

Results

Demographic characteristics

Demographic characteristics are displayed for the combined sample and each group individually in Table 2, including the mean age, gender, laterality, and mean verbal memory scores. The mean verbal memory scores were derived from the Rey Auditory Verbal Learning Test (RAVLT) raw scores by summing up scores from trials 1 to 5 (i.e. RAVLT immediate summary score). No statistically significant differences in age, *F (*2,44*)* = 0.62, *p* = 0.54, or verbal memory scores, *F (2,*44*)* = 0.471, *p* = 0.63, were found between the groups.

Table 2

Demographic Characteristics

	Total (N=47)	Sham (N=17)	6 Hz (N=12)	Ind. Theta (N=18)
Age	71.72+/-7.94	72.53+/-8.84	69.50+/-9.11	72.44+/-6.22
Gender (Male/Female)	15/32	9/17	2/12	4/18
Laterality (left-/right-handedness)	4/43	3/14	1/11	0/18
RAVLT Scores	31.11+/-8.56	31.41+/ 8.22	29.08+/-8.79	32.17+/-9.02

Note. Data is presented as mean +/- standard deviation for the whole sample and the three stimulation groups separately.

Default Mode Network Connectivity

The results of the 3x2 mixed ANOVAs testing for a differential effect of stimulation type on the clustering coefficient and average path length within the DMN (and FPN) are displayed in Table 3. The analyses revealed no significant interaction between stimulation type (i.e. individual, 6Hz, sham) and time of resting-state measurement (i.e. pre, post) for the clustering coefficient or average path length. This suggests that sham tACS, individual theta tACS, and 6Hz tACS did not significantly differ in their impact on graph metrics between pre-stimulation and post-stimulation measurements. This lack of interaction was observed for the combined network and at each DMN node (ROI) individually (Table 3). Post-hoc paired sample t-tests showed no significant differences in clustering coefficient or average path-length on the network level between pre- and post-measurements for any stimulation type (Table 4). Thus, tACS did not effectively modulate DMN resting-state functional connectivity in patients with aMCI, measured by graph metrics of local integration and segregation.

Frontoparietal Network Connectivity

The 3x2 mixed ANOVA revealed no significant interaction between stimulation type and time of resting-state measurement within the FPN for the clustering coefficient and average path length (Table 3). Again, this result was observed for the combined network and at each node individually. For the average path length, the interaction term reached significance at an alpha-level of 0.05 for the FPN combined and the R-LPFC but did not survive FDR-correction. Thus, the effect of tACS on graph metrics in the FPN was not significantly different form sham when accounting for multiple comparisons. On the network level, post-hoc paired sample t-tests showed no significant differences in clustering coefficient or average path length between pre- and post-measurements for any stimulation type (Table 4). In the sham condition, the t-tests reached significance for the clustering coefficient and average path length at alpha= 0.05 but turned insignificant following FDR-correction.

Table 3

Results of 3x2 ANOVA testing interaction effect on Graph Metrics in DMN and FPN on network and node/ROI level

RS-Network	Graph Metric	ROI	DF	F	P
Default Mode Network	Clustering Coefficient	Whole Network	2,44	0.042	0.960
		MPFC	2,20	0.435	0.653
		L-LP	2,41	0.027	0.973
		R-LP	2,42	0.171	0.844
		PCC	2,42	0.936	0.400
	Average Path Length	Whole Network	2,44	1.163	0.322
		MPFC	2,35	2.615	0.087
		L-LP	2,44	1.471	0.2408
		R-LP	2,44	0.3212	0.272
		PCC	2,44	0.031	0.970
Frontoparietal Network	Clustering Coefficient	Whole Network	2,32	2.651	0.086
		L-LPFC	2,18	1.509	0.248
		L-PPC	2,25	0.649	0.513
		R-LPFC	2,14	0.600	0.564
		R-PPC	2,28	1.844	0.177
	Average Path Length	Whole Network	2,43	2.234	0.049
		L-LPFC	2,39	1.980	0.152
		L-PPC	2,41	1.893	0.164
		R-LPFC	2,40	4.631	0.016
		R-PPC	2,41	0.941	0.400

Note. ** significance after correcting for false discovery rate (FDR)

Table 4

Results of two-sided sample t-tests comparing graph metrics pre- to post-stimulation per network and condition

RS-Network	Graph Metric	Condition	DF	T	P
Default Mode Network	Clustering Coefficient	Sham	16	0.62	0.542
		Ind-Theta	17	1.08	0.294
		6Hz	11	0.34	0.738
	Average Path Length	Sham	16	-1.33	0.203
		Ind-Theta	17	-0.89	0.386
		6Hz	11	0.71	0.491
Frontoparietal Network	Clustering Coefficient	Sham	11	-2.54	0.027
		Ind-Theta	13	1.00	0.338
		6Hz	8	-0.35	0.735
	Average Path Length	Sham	16	2.851	0.012
		Ind-Theta	16	-0.941	0.361
		6Hz	11	0.26	0.799

Note. ** significance after false discovery rate (FDR) correction. Uncorrected p-values are displayed.

Discussion

This is the first study to investigate the effects of theta transcranial alternating current stimulation (tACS) on resting-state functional connectivity (rs-FC) in individuals with amnestic mild cognitive impairment (aMCI) using a graph theoretical approach. Specifically, the study aimed to determine whether repeated administration of individualized theta frequency tACS, 6Hz standardized theta tACS, or sham stimulation over the left frontal and parietal cortex could induce changes in rs-FC within the default mode network (DMN) and frontoparietal network (FPN). It was hypothesized that both active stimulation groups would show greater changes in rs-FC from baseline to post-stimulation compared to the sham group. Additionally, individualized theta tACS was expected to have a larger effect on rs-FC than tACS delivered at a standardized 6Hz frequency.

The results of the statistical analyses were insignificant, suggesting that the graph metrics of rs-FC did not change between the pre- and post-stimulation measurements within the DMN and FPN in any of the stimulation conditions. Considering these results, both hypotheses were rejected. Thus, tACS at an individualized and a standardized theta frequency did not modulate rs-FC in patients with aMCI.

Interpretation of Results

This study adds to a growing body of literature investigating the neural underpinnings of tACS effects. The application of theta tACS, under the parameters tested in this study, does not appear to modulate rs-FC within key brain networks in patients with aMCI. These null findings are in contrast with previous studies conducted in healthy, aging, and clinical populations demonstrating that theta tACS modulates fMRI and EEG measures of rs-FC (Aktürk et al., 2022; Jones et al., 2022; Khan et al., 2023). In some of these studies, stimulation induced connectivity change was predictive of improvements in cognitive functioning, while in others, connectivity change in the stimulation group was not related to cognitive improvements. Considering these discrepancies and the lack of a significant effect of tACS on rs-FC in the current study, it is difficult to draw conclusions about the role of functional connectivity changes in cognitive improvement through tACS treatment. Specifically, how tACS modulates functional connectivity and how such changes may relate to cognitive improvements remains unclear. However, these findings highlight the challenge of stimulating neural networks in individuals with aMCI, where cerebral atrophy causes an increasing disconnection of brain networks involved in cognition (Liang et al., 2011; Tabatabaei-Jafari et al., 2015). Possibly, standard tACS protocols need adjustments to be effective in populations with neurodegenerative diseases, which would have significant implications for future clinical applications. It is important to consider several factors that could account for the non-significant effect of theta tACS on rs-FC in aMCI patients. These factors can be broadly categorized into the study population characteristics, nature of the intervention, connectivity analysis scope and methodological limitations.

Study population Characteristics

As mentioned above, it is possible that brain networks altered by aMCI brain pathophysiology respond differently to tACS stimulation compared to healthy brains. Indeed, a recent study found age-related variations in DMN connectivity following intermittent theta-burst stimulation (iTBS) (Abellaneda-Pérez et al., 2019). Among younger adults, connectivity to distal DMN regions increased after iTBS, while for older adults more proximal connectivity increased. Critically, greater brain integrity, higher cognitive baseline performance, and education of participants in the old age group was associated with brain responses resembling those observed in the younger group. Given the compromised brain integrity and low cognitive baseline associated with aMCI, it is likely that the brain networks of individuals with aMCI do not respond to tACS stimulation in the same manner as those of younger-healthy participants, who are often the subject of such studies. Therefore, stimulation protocols may need to be adjusted for this patient population. In line with this, Jones et al. (2023) suggested increasing the stimulation intensity to account for brain atrophy in individuals with aMCI.

The findings by Abellaneda-Pérez et al. (2019) lead to another consideration. Specifically, the heterogeneity in the study population may have obscured the effects of tACS on functional connectivity on the group level. It is plausible that certain sub-populations in the study sample (e.g. lower disease progression, higher brain integrity, higher education, higher baseline cognitive funcitoning, etc.) responded with changes in rs-FC to the stimulation, while others did not. It could be interesting to stratify the sample by demographic characteristics and clinical variables to distinguish responders from non-responders. Sub-populations of aMCI patients for whom tACS treatment is more effective could be identified. However, considering the small sample sizes in the current study, this was not possible.

Taken together, both the heterogeneity and the brain atrophy associated with the study population could potentially account for the null findings.

Nature of the Intervention

Given the previous considerations, it is crucial to examine the nature of the intervention in this study. The efficacy of tACS is highly dependent on stimulation parameters such as frequency, intensity, duration and electrode montage. The parameters used here may not have been optimal for entraining endogenous brain rhythms and modulating neural networks in the aMCI population. For instance, the protocol utilized by Khan et al., (2023), which showed an effect of theta tACS on rs-FC in healthy participants, used slightly different parameters. The stimulation was applied in fewer sessions but with a longer duration of 25 minutes per session, compared to 15 minutes in the current study. Additionally, stimulation was delivered at a higher current of 1.5 mA, as opposed to 1 mA in this study. These differences suggest that theta tACS might need to be applied at higher intensities and longer durations to effectively modulate rs-FC in patients with aMCI.

Connectivity Analysis Scope

The previous sections addressed potential reasons why tACS under the parameters in this study did not induce changes functional connectivity, assuming there were no effects on connectivity. However, it is possible that the scope of the functional connectivity analysis was not adequate to detect changes that were actually present. Specifically, the current approach to analyzing functional connectivity may have limited the ability to detect the full range of stimulation effects, as choices were made in order to limit the focus of the analysis.

The first aspect that may have narrowed the scope of observable changes is the focus on within-network connectivity. Complex brain and cognitive functioning is driven by integrated processing in several interacting networks (Vatansever et al., 2015). By concentrating on the interactions between nodes belonging to the same network (i.e. within-network connectivity), important changes in connectivity occurring between different networks or between the stimulated networks and other brain regions may have been overlooked in the current study. Support for this idea is provided by Khan et al., 2023 who administered multiple sessions of individualized theta tACS to the left dorsolateral prefrontal cortex (L-DLPF) of healthy individuals during arithmetic learning. They observed increased connectivity between the precuneus, a brain region involved in cognition and arithmetic learning, and the FPN in the stimulation group. Additionally, increased connectivity between the FPN and DMN was demonstrated. This indicates that tACS could potentially modulate inter-network connectivity patterns which the analysis of within-network connectivity in the current study did not capture.

Second, the emphasis of this study on static resting-state connectivity may have hindered the identification of more state-dependent tACS effects on active cognitive processing. The modulation of functional connectivity may be more pronounced when assessing network functioning during active task states as compared to the resting state, especially since stimulation was delivered during active working memory processing. While resting-state and task-based networks overlap spatially, functional interactions and activity at rest may qualitatively differ from active processing (Vatansever et al., 2015). For example, Vatansever et al., (2015) demonstrated that during task performance, transient interactions occur between parts of the DMN and brain regions that do not interact at rest (Bola & Borchardt, 2016). More precisely, default mode regions dynamically switched community membership during performance of the n-back task and reorganized to interact with other networks (Vatansever et al., 2015). This reorganization was related to working memory performance. Similarly, in the current study aMCI patients received stimulation while performing the n-back task. It is possible that the stimulated networks, particularly the DMN, exhibited connectivity changes within the active stimulation groups only during task performance and while reconfiguring to interact with other brain regions. However, the analysis of static resting-state connectivity employed in this study is insufficient to capture such changes in dynamic task-based connectivity.

Lastly, the graph theory analysis method employed in the current study may not have been sensitive enough to capture changes in functional connectivity. In fact, heterogeneity in functional connectivity analysis methods employed in different studies appears to affect study outcome and the reproducibility of results (Canario et al., 2021). For graph theory in particular, Phillips et al., (2015) demonstrated that the method of graph creation can determine the size and direction of group differences in graph measures. In sum, changes in functional connectivity may have been overlooked because of the emphasis on within-network connectivity and resting state connectivity or the graph theory method used.

Limitations

This study has several methodological limitations which should be considered when interpreting the results, some of which have been discussed above (i.e. cortical atrophy, heterogeneity in the study population, functional connectivity analysis scope). First, a significant methodological limitation that restricts the conclusions that can be drawn from this study is the small sample size within each group (i.e. 18, 12, and 17). The number of participants per group is smaller than the already low sample size common in cognitive neuroscience studies which is between 23-24 (Szucs & Ioannidis, 2020). Small sample sizes limit the statistical power, reducing the likelihood to detect a small effect. Thus, the low number of participants in each group could have contributed to the null findings, as the study may have lacked adequate power to detect a small change in functional connectivity induced by tACS. Recommendations for appropriate sample sizes in neuroimaging studies propose between 40 and 80 participants to achieve reliable results (Geuter et al., 2018; Sideridis et al., 2014). Based on these recommendations, at least 120 participants should have been recruited for this study. Another methodological limitation is related to the use of rs-FC fMRI repeated measures designs, such as the one employed in the study. Intraindividual variability in resting state network connectivity is a limitation of fMRI repeated measures designs because it reduces the test-retest reliability of rs-fMRI measures. (Canario et al., 2021; Harrison et al., 2008; Specht, 2020). Variabilities in rs-FC within the same individual are related to psychological and environmental factors, such as mood or time of the day (Blautzik et al., 2013). Additionally, intraindividual differences in resting-state activity appear to be location specific, showing the highest variation in areas related to working memory and executive functioning, which were the subject of this study (Canario et al., 2021; Chen et al., 2015). It is likely that conditions that affect network functioning, such as aMCI, increase the variability in resting state activity compared to healthy brains. Especially considering the small size of the intervention groups, it is possible that intra-individual variability in resting state measures masked modulatory effects of tACS on resting state connectivity in this study. Next, while the stimulation frequency was individualized, this study did not account for individual differences in brain anatomy to increase the precision at which brain networks are targeted. This variability can reduce the consistency of neuro-modulatory effects, which is to say that it is unclear if, and unlikely that the same brain regions were targeted at equal field strengths across participants. Indeed, a recent study found that variability in the effects of 6Hz tACS above the prefrontal cortex was related to individual differences in neuroanatomy (Zanto et al., 2021). Additionally, the electrode montage utilized in this study targeted a relatively large cortical area. This results in a lack of specificity and the possibility that non-target brain areas were stimulated which could be prevented by utilizing more targeted stimulation techniques such as transcranial temporal interference stimulation (Grossman et al., 2017; Khan et al., 2023).

Future Directions

Multiple recommendations for future research can be derived from the considerations and limitations discussed above. Future studies should aim to recruit larger samples to improve statistical power and increase the likelihood of detecting small effects. Additionally, a larger sample would allow to control for the heterogeneity in the clinical sample population by introducing covariates and stratifying the sample by factors such as baseline cognitive functioning, progression of disease and neuropathology, as well as education. This could lead to the identification of responders and non-responders and patients that are more sensitive to the neuro-modulatory effects of tACS. Next, future studies should broaden the functional connectivity analysis scope, moving beyond within-network connectivity to investigate rs-FC changes between different networks and across the whole brain. Possibly, the cluster-analysis approach utilized by Khan et al., 2023 could be replicated in the aMCI population.

Additionally, the effect of tACS on task-based functional connectivity and network reorganization in individuals with aMCI should be investigated in future studies by utilizing advanced dynamic functional connectivity analysis approaches. Observing task-based functional connectivity may also advance our understanding how network disruptions in aMCI give rise to cognitive dysfunction. Another approach for future studies could be to integrate rs-fMRI and EEG approaches. Examining how online EEG measurements of entrainment translate into functional connectivity changes could advance our understanding of the relationship between tACS and brain network functioning. Future studies should investigate different theta tACS protocols, focusing on stimulation parameters that have been proven successful, such as the one utilized by Khan et al., (2023). In general, it appears that stimulation should be delivered at higher amplitudes and over longer durations. Additionally, computational modelling approaches should be employed to simulate the electric field distribution in brains with cerebral atrophy, which may help to identify optimal stimulation parameters for the aMCI population. Moreover, stimulation parameters should be further individualized to improve the stimulation specificity. By using neuro-navigation techniques and structural brain images, target regions could be identified, and electrode placements tailored to the individual to account for inter-individual differences in brain morphology, ensuring that the same areas are stimulated across participants. Lastly, stimulation tools with higher stimulation specificity, such as transcranial temporal interference stimulation, should be investigated to target specific brain networks, such as the DMN and FPN, more precisely.

Conclusion

In conclusion, this study is the first to investigate the effects of theta transcranial alternating current stimulation (tACS) on resting-state functional connectivity (rs-FC) in patients with amnestic mild cognitive impairment (aMCI). Previous studies indicate that tACS has the potential to improve cognitive functioning by modulating communication in brain networks that are increasingly disconnected during the progression from healthy cognitive aging to aMCI, and finally Alzheimer's disease. In contrast, neither standardized nor individualized tACS modulated rs-FC within the default mode network (DMN) and frontoparietal network (FPN) in patients with aMCI. The findings suggest that, under the parameters tested, theta tACS does not modulate functional connectivity in aMCI patients and tACS protocols may need adjustments to be effective in the aMCI population. Several interpretations of these findings were considered, discussing the importance of the functional connectivity analysis, methodological limitations, the heterogeneity of the sample population, and stimulation parameters. Future research should focus on these areas to advance our understanding of the neural underpinnings of tACS effects in the aMCI population and identify optimal stimulation parameters.

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