Gamma Induction Using Transcranial Alternating Current Stimulation in Alzeihmer's Disease

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Abstract

Thanks to technology and advances in the medical field, our population's lifespan continues to lengthen. As a result, aging related neurodegenerative disorders become more prevalent. Manipulating brain oscillations is a potential solution to cognitive impairments that result from these disorders. This form of manipulation can be accomplished with the use of transcranial alternating current stimulation (tACS). The use of noninvasive stimulation technology to explore brain activity provides greater insight into the role brain oscillations have in cognition, specifically, into the role of gamma oscillations in Alzheimer's Disease (AD) patients. AD is characterized by alterations in inflammatory processes and depositions of proteins like Aβ and tau, as well as alterations in levels of gamma. Recent studies have shown that inducing gamma in mice modulates microglial activities and thus modified the inflammatory processes.

In this study we researched the safety and feasibility of the first in-human study that translates these findings using tACS at 40Hz to induce gamma oscillations through a 10 day period in AD. Using MRI and PET imaging, we were able to optimize the intervention and target regions with greater Aβ accumulation. Fifteen participants were separated into 3 groups with slightly differing stimulation protocols, they all had daily recordings of changes in EEG, cognition, adverse effects, and safety. Gamma induction was compared pre and post stimulation and the results showed that gamma was induced post-stimulation, as well as the possibility of additive effects throughout stimulations. Additionally, N-back tasks results suggest the protocol was beneficial to cognitive processes with improvements to declarative memory and a reduction in the P200 latency in the N-back task, indicating quicker working memory processing. There

was a correlation in declarative memory and gamma connectivity in the bi-hemispheric temporal stimulation which indicates that gamma contributes to an improvement in cognitive performance. Overall, there were no noteworthy adverse effects and minimal levels of attrition.

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Table of Contents

List of Tables

List of Figures/Graphs

Chapter I

Introduction

Alzheimer's Disease Background

Alzheimer's disease (AD), is a neurodegenerative disease that is characterized by abnormalities in beta-amyloid and tau proteins, alongside disruption in memory, attention, learning, and a decline in overall cognitive domains (Masters et al., 2015; Ferrucci et al., 2008). It is an irreversible and progressive brain disorder that causes dementia and permeating neurodegeneration. What differentiates AD from other neurodegenerative or dementia causing diseases is the abnormal B-amyloid (Aβ) and neurofibrillary tau protein deposits (Jack et al., 2018). Additionally, AD is currently known to be the most common cause of dementia. The disease also leads to a decline in behavioral and social skills all of which can inhibit the ability to properly function independently in everyday tasks. Evidence shows that the buildup of the aforementioned proteins contribute to memory deficits and AD, therefore interventions that reduce these proteins can potentially make a substantial impact.

Diagnosis & Treatments

Separate guidelines for diagnosis rely on preclinical, mild cognitive impaired, or dementia stages of the disease (Jack et al., 2018). Diagnosis can be based from syndromal (e.g. clinical symptoms) to a biological diagnosis (e.g. usage of biomarkers). The available

pathological processes are able to be documented through post mortem exams or biomarkers (Jack et al., 2018).

Current AD treatments mostly target either poor clearance mechanisms or the abnormal generation of Aβ. The majority of drugs have been unsuccessful or have caused severe side effects (Canter et al.,2016). Current treatment options for AD are mostly pharmacological with the most common being drugs like Cholinesterase Inhibitors and Memantine which have possible side effects.

Definition of Terms

"Amyloid plaques": Clumps of beta amyloid that are hard and insoluble and thus can destroy the connection of nerve cells. This impacts behavior, memory and thinking and are usually found in the brain of a patient with AD. These plaques can form throughout time as the enzyme that slices the amyloid precursor protein into beta amyloid sometimes slices into larger strands that can't dissolve. These resulting strands often stick and start clumping together resulting in plaques.

"Default mode network": A brain network known for activating when a person is at rest, or not focused on the surrounding world. There's been research indicating that the network has a negative correlation with attention networks and AD patients tend to have disruptions in the DMN.

“Dementia”: This is the terminology used as a description for conditions and diseases that are characterized by a decline of memory, social abilities, and thinking. These symptoms are usually severe enough that they interfere with the patients daily life activities.

“Gamma frequency”: A gamma wave is a specific oscillation that normally averages at a frequency around 40 hertz (can range from 25-100 hertz). Throughout time there have been correlations between gamma oscillations and awareness, learning, and other cognitive abilities.

“Microglia”: These are cells that can mediate immune responses in the nervous system. They are derived from mesoderms and act as macrophage, clearing out the waste and debris in neurons through phagocytosis.

“Neurofibrillary tangles”: Inside the nerve cells there are insoluble fibers called neurofibrillary tangles. The tangles are made from tau protein that form parts of microtubules which helps move nutrients and other substances throughout the cell.

“Brain/Neural oscillations”: In the brain there are neural oscillations that are rhythmic in electrical activities, they are rhythmic and generated constantly as a result of stimuli.

“Phosphorylated Tau protein”: Tau proteins stabilize the microtubules in our cells, most abundantly in neurons. In AD these proteins become defective and can’t continue to stabilize the microtubules in the cells correctly. A buildup of this protein is caused by abnormal tau kinase enzyme activity which leads to the misfolding and clumping of tau and the formation of neurofibrillary tangles.

“Transcranial alternating current stimulation (tACS)”: Is a noninvasive form of brain stimulation that oscillates electrical currents using electrodes in the scalp in order to manipulate brain activity.

“Tauopathies”: is a term given to neurodegenerative disorders usually showing abnormal activity of tau proteins.

“Oscillatory rhythmic activity”: Neuronal populations generate electrical activity with differing frequency bands. Brain rhythms are gauged according to phase, frequency and power (or amplitude).

“Network hypersynchrony”: This occurs when there is excessive synchronization between neurons.

“Neuronal synchronization”: Is the degree to which neuronal activities are related to one another.

Pathophysiological Framework

For many years research on AD has mostly centered around neurons, recently scientists have found the importance of immune cells in the disease. The immune system in our brain begins to disintegrate with age which sets off a series of events that allow the accumulation of harmful proteins like Aβ and tau (see Figure 1). This clogs the connections between neurons and thus the symptoms of AD arise. Initially Aβ was suggested to be a main component to AD, however, throughout time the typical treatment options provided to reduce Aβ have not worked

towards the goal of restoring or improving the cognitive symptoms in the disease, indicating that the decline in cognition might be due to a more complex pathophysiology.

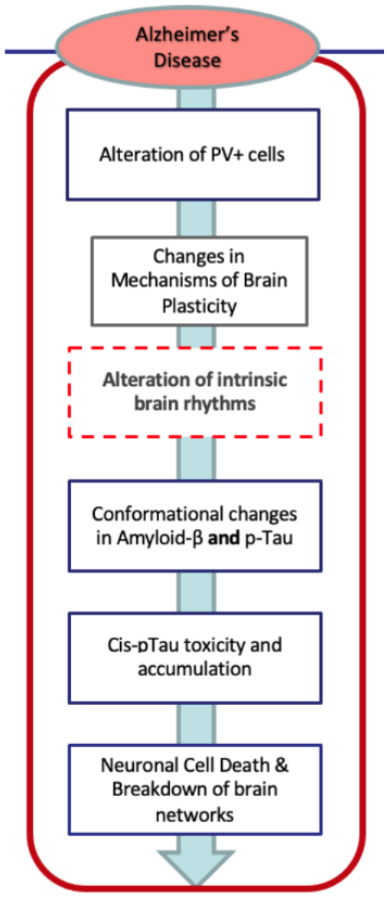

Figure 1. Pathophysiological Framework

Alzheimer's has a complex pathophysiology that include a set of events that lead to neuronal cell death and the breakdown of brain networks. Although the exact inception to the chronology of events is yet to be determined, we are aware of the role alterations of intrinsic brain rhythms have in the disease.

Proteinopathy

Aβ results from cleaving an integral membrane protein called an amyloid precursor protein (APP). APP is cut into smaller fragments by enzymes, the fragments created are soluble amyloid precursor protein and Aβ. Soluble APP is said to have growth promoting properties while Aβ may be involved in the plasticity of neurons. In a normal brain the alpha-secretase enzyme cleaves APP into sAPP-alpha and into CTF83, which is further cleaved by gamma-secretase into AICD which acts on the transcriptional regulation and neuroprotective pathways while sAPP-alpha is what allows for normal synaptic signaling which in turn leads to plasticity, neuronal survival as well as memory function and learning. .

In an AD brain, a beta-secretase (another potential biomarker for AD) enzyme cleaves the APP into sAPP-beta and CTF99. The gamma-secretase still cleaves the CTF99 fragment into AICD but it also yields an Aβ 40/42 fragment which is involved in several downstream pathways related to AD. The Aβ 40/42 associates with ApoE, this causes an aggregation of β oligomers later resulting in amyloid plaques (Stutzmann, 2020).

Aβ peptides can become very harmful to the brain, as it can cause neuronal apoptosis and in the long term can lead to oxidative damage in protein and DNA, dysregulation in calcium and injury of cellular organelles. All of these outcomes can result in eventual cell death and can ultimately result in dementia and memory loss. The consequences throughout time have various notable symptoms such as cognitive decline. Although prior to this other deficits can be underworks such as in circuit function, cognition and synaptic plasticity (Palop et al., 2010). In a study using mice, sleep deprivation promoted Aβ deposition while good sleep promoted

clearance, showing that brain state can alter protein depositions (Roh, J. H. et al., 2012; Kang, J. E. et al., 2009; Xie, L. et al., 2013).

The buildup of Aβ (Canter, 2016) in the brain can contribute to the decrease in synapses and alteration in overall neuronal activity. This can lead to a disruption in neural circuits and thus network dysfunction and a decline in cognition. There are various studies that provide evidence for the role of Aβ such as one on familial genetics. AD cases resulting from mutations in APP or PSEN1/2 alter the cleavage processings for the precursor protein towards the production of Aβ peptides (Hardy, 1997). Heritable alterations resulting in greater production of the toxic plaques is likely to result in AD. On the opposing side, an APP variant that does not increase the production of Aβ can provide protection against developing the disease (Jonsson, T. et al., 2012). Other genetic risk factors include mutations in the APOE4 gene. This gene can lead to more than double increase for having AD, a patient that carries this gene has greater evidence of the protein aggregation in comparison to a patient who does not have APOE4 (Morris, J.C. et al., 2010).

Intracellular characteristics of AD include aggregations of tau proteins, neurofibrillary tangles. The amount of these tangles has a strong correlation to the progression of AD (Hyman, B. T. et al., 2012). Tau protein aggregations occur as a result of hyperphosphorylated tau assembly into filament pairs (Hyman, B. T. et al., 2012). In a normal brain, dephosphorylation regulates the necessary tau levels, in contrast, and AD brain the calcium influx and hyperactivity cause abnormal tau phosphorylation. The aggregation results from different kinases and dysfunction of the protein phosphatase 2A (PP2A) which regulated appropriate levels of tau

which trigger the hyperphosphorylation and leads to aggregation and insolubility of the protein (Sontag, 1996; Cruz, 2003).

It is worth noting that AD should be considered a disorder of the brain, meaning that systemic factors are at a constant interaction with the brain and modifying the disease processes and that the interactions between the brain and periphery are very important in the development of AD. This idea was brought up by Jun Wang et al, as they looked at AD systemically. Some symptomatic abnormalities found in AD include cardiovascular disease, disorders in systemic immunity, hepatic dysfunction, metabolic disorder, blood abnormalities, respiratory disorder, sleep disorders, renal dysfunction, microbiota disturbance and infection, and system inflammation.

Inhibitory Interneurons & Network

Synaptic activity is dependent on Aβ concentrations, when low amounts of Aβ are present there is a resulting excitatory activity being promoted, and vice versa. Large increases of Aβ can lead to impairments in long term potentiation of the synapse strength and lead to long term depression (Walsh, D. M. et al., 2002; Li, S. et al. 2009; Hsieh, H. et al. , 2006). Aβ can create an imbalance of inhibitory and excitatory activity through its effect on inhibitory interneurons.

GABAergic interneurons control network synchronization and brain oscillations (Buzsaki, 2004). The inhibitory synaptic input of GABAergic interneurons along with the

excitatory input of principal neurons result in oscillatory rhythms which lead to the timing of pyramidal cells firing (palop). Inhibitory interneurons like parvalbumin-positive (PV+) interneuron fire mainly during encoding related states (Lapray, D. et al., 2012; Fu, Y. et al., 2014). PV+ cells make up a bit less than half of all inhibitory interneurons, they are also the main inhibitors to pyramidal cells (Palop, 2020). Additionally, PV+ cells are electrically coupled through dendritic junctions, forming a coordinated network. This electrically coordinated network permits synchronous modulation of pyramidal cells (Hestrin, 2005). Studies done on mice showed an increase in gamma when the firing rate of PV+ cells was increased, in contrast, increasing pyramidal cell firing rate resulted in increasing only low frequency oscillations (Sohal, 2009; Cardin, J. A. et al., 2009). Patients with AD usually have an decrease in higher frequency oscillations like gamma power and an increase in lower frequency oscillations (Nimmrich, 2015).

As previously mentioned, during AD these interneurons and networks are changed. Recent findings on this topic have provided support for the modulation of interneurons having a positive effect on cognitive functions for AD patients (Verret, L. et al., 2012; Southwell, D. G. et al., 2014; Hunt, 2013; Tong, L. M. et al., 2014). Inhibitory interneuron functioning is crucial for proper gamma, oscillation frequencies, and neuronal firing activity.

Microglia work with inhibitory neurons oscillating at a specific frequency. These inhibitory neurons oscillate at their frequency, working like an alarm sound. When that alarm sound goes off our brains shift into defense mode clearing out the harmful proteins and attempts

to reduce the protein aggregations. As we age we lose inhibitory neurons and those with Alzheimer's lose them even more quickly.

Microglia are activated as an immune response and these cells then form associations with the amyloid plaques. Immune effector cells in the brain such as microglia, maintain the brain's homeostasis while also inhibiting infections and other damage. When these immune cells are activated and form associations with amyloid deposits, they mount immune responses to amyloid beta and migrate to areas of amyloid deposition (Mandrekar-Colucci, 2010).

Maintenance of the brain's cellular environment includes the clearance of debris by microglia in order to maintain healthy connectivity and signalling. There are a number of ways the body clears out Aβ, including phagocytosis, macropinocytosis, endocytosis, proteolytic degradation, and through efflux of Aβ to the peripheral circulation. Specifically, microglia analyze their surroundings for unwanted and possibly harmful debris (Harris, J. A. et al., 2010). Once detected, the debris is removed by the microglia through pinocytosis and phagocytosis processes.

Network modifications such as activation/deactivation deficits, hypersynchrony, and abnormalities in oscillatory rhythms are linked to successfully retaining new information and to the encoding of memories. In AD, certain neuronal networks that support cognition become altered and findings suggest that these alterations in network activities can contribute to the impairments seen in AD and that we can manipulate these networks in patients with risk of developing AD. Resting networks are characterized by slow frequencies with high amplitude fluctuations while active networks have desynchronization with fast frequencies and low

amplitude fluctuations. The desynchronization with lower amplitude fluctuations allows the neurons to respond better to external inputs (Poulet, 2008).

If Aβ accumulation persists throughout time there will be neuronal hyperactivity caused by the synaptic loss and disinhibition of excitatory cells in regions of the brain most correlated with memory and learning (Vossel, K. A. et al., 2013; Busche, M. A. et al., 2015). Using positron emission tomography (PET) it's been possible to trace the brain networks that appear to be more susceptible to AB. Longitudinal studies tracing Aβ have found that there is noteworthy aggregation in the neocortex, specifically in the default mode network (DMN) (Greicius, 2005; Buckner, 2005), and support the predictive abilities of Aβ for cognitive declines (Jack, C. R. et al., 2010;Forsberg, A. et al., 2008). The default mode network is composed of regions activated when passive thinking, planning, remembering are taking place, therefore a constant activation or faulty inhibition of this area can become concerning. MRI scans have shown decreased connectivity in the DMN suggesting the vulnerability of this network to AD processes (Greicius, 2005). In a normal brain, attention-related cognitive tasks show a large deactivation in the collective brain areas known as the DMN (Boyatzis,2014; Raichle, M. E. et al, 2001). Interneuron dysfunction accompanied by aging results in failure to inhibit the DMN.

Brain networks appear to be a prominent target towards restoring memory. Specifically, brain stimulations that manipulate network activity have shown improvements in memory (Laxton, A. W. et al, 2010; Kuhn, J. et al., 2015). Non-invasive brain stimulation has altered protein expression when done in animal models (Sankar, T. et al., 2015) and optogenetic studies done to excite cells throughout the hippocampus had positive outcomes on memory and learning

(Roy, D. S. et al., 2016). These studies support that intervention in the circuit and network level can restore memory, circuit integrity, and cell health.

Gamma Oscillations

Manipulating brain oscillations has provided a promising route to improve cognition. Neural circuits and networks are able to be manipulated by modulating the synchrony of their components. A network's abnormal synchrony or oscillation can contribute to cognitive abnormalities in AD. The onset of these abnormalities occur many years prior to the clinical onset suggesting that these abnormalities may predict AD pathology (Palop, 2020) .

Specifically, gamma waves are those produced during heightened perception, learning, problem solving tasks, and cognitive processing. tACS can reproduce the gamma oscillations that in other research have been shown to modulate the activity of microglia in mice (Iaccarino et al., 2016), modifying the inflammatory brain processes leading to greater clearance of the harmful proteins associated with AD (Iaccarino et al., 2016). Using tACS we can induce these gamma oscillations and create long lasting changes in the p tau and amyloid plaque in AD patients by assessing the levels through PET images.

Neuronal ensembles have a firing range of frequencies from 0 to 300 Hz, these numbers indicate the amount of cycles occurring each second during oscillation. These oscillations are separated into different frequency bands starting with delta which stands at 1-4Hz and gamma at 35-55Hz. Gamma can be divided into low and high levels where lower gamma is at a 35-45 Hz

range and higher gamma is 45-55Hz (8). The greater the amount of neurons that have synchronized their firing rates at a specific band, the greater the power of that band.

As was previously mentioned, oscillatory frequencies range from 0-300Hz. Some interneurons show high frequency oscillations of 30-150Hz, or gamma (Jensen,2007; Viney, T. J. et al., 2013; Tukker, 2007), therefore during a period of higher gamma power, there will be greater firing of action potentials in these interneurons and they will have greater synchronization with gamma (Viney, T. J. et al., 2013). Gamma power increases during memory encoding, this increase predicts the success of memory formations (Uhlhaas, P. J. et al., 2009; Matsumoto, J. Y. et al., 2013; Sederberg, P. B. et al.(A), 2007; Sederberg, P. B. et al.(B), 2007; Jensen, 2007; Yamamoto, 2014). AD patient's have reductions in gamma power (Herrmann, 2005).

A study by Iaccarino et al. using an Alzheimer's mouse/mice model showed that we can achieve protein clearance using gamma inductions. The results showed that twice as many microglia in a group being given 40 Hz stimulations decreased hippocampal Aβ compared to the control groups as seen in immunohistochemistry. Providing evidence to the idea that 40Hz of oscillations can induce a neuroprotective response that recruits microglia as well as neurons.

These oscillations can be studied in subjects as they conduct a specific task or when they are in a resting state. Studying the resting states shows the fundamental oscillatory profile of that subject, while the task specific study shows the changes in oscillations that have been caused in response to the task.

The role of oscillations throughout neural processing can be supported when looking at the phylogenetic preservation, Hebbian plasticity, and emphatic couplings in neural populations. When looking at EEG studies, changes in amplitude of frequency bands indicate differing cognitive functions and brain states. For example, there is an observed increase in alpha power throughout the occipital cortex as a result of a subject's eyes closing shut (Wang ,2010). When a subject is asleep Delta is the main band present (although depending on the stage of sleep) (Buzsaki, 2014), Theta in memory function and emotional regulation (Knyazev, 2007), Alpha in neural operations during absence of sensory input (Palva, 2007), and Beta is associated with motor control and varying other functions (Engel, 2010). Gamma, as a faster frequency, is linked to attention, sensory response, spike timing, and synchrony during the formation of memories (Bouyer, 1981; Cardin, J. A. et al., 2009; Carr, 2013). Gamma increases during tasks requiring working memory (Chen, 2014) and memory encoding in humans as well as in mice (Colgin, 2016; Yamamoto,2014).

Recordings have provided support for the fact that changes in gamma can predict cognitive performance. Sederber et al showed that the greater the gamma oscillations in the temporal and frontal cortex predicts improved encoding of verbal memory (Sederberg, P. B. et al., 2007). Additionally, Womelsdorf et al gave evidence that gamma synchronization could predict the reaction time necessary in a detection task, providing support of gamma's involvement in attention processes.

Studies have investigated the origin of these rhythms. It has been suggested that the rhythms are the cause of the overall activation of pyramidal neurons (Fitzgibbon, 2004). More recently it has been proposed that the activation comes from gabaergic interneurons (Chen,

2014). Commonly seen in these oscillations are the parvalbumin positive (PV+) basket cells, logical considering how other interneurons are usually excitatory to molecules that void gamma (Mably, 2018).

Brain oscillations provide a common language for noninvasive, invasive and modeling studies as they can be observed throughout distinct spatial levels. Optogenetic manipulation of gamma oscillations in mice models of AD showed recruitment of neuronal and glial responses (Cohen, 2017). This manipulation can be accomplished with the use of transcranial alternating current stimulation (tACS).

tACS and Cognition

Transcranial Alternating Current Stimulation

Transcranial alternating current stimulation (tACS) is a type of transcranial electrical stimulation (tES) technique which is a noninvasive form of brain stimulation that uses electrical currents to stimulate neuronal activity in parts of the brain nursing electrical currents. During the treatment, participants have electrodes strategically placed into a cap on their heads while the electrical current is traveling to and from the two locations. This form of stimulation uses a low intensity sinusoidal current passed through an electrode placed on strategic parts of the scalp. The resulting electric field then causes an electric field to oscillate through the desired brain regions. The ability for this noninvasive stimulation to be able to entrain an oscillation and neurons makes this technology very useful for neuromodulation. tACS has the ability to

reproduce the gamma oscillations which can modulate the activity of microglia that in turn modify the inflammatory brain processes which can then lead to greater clearance of the harmful proteins (Aβ and p-tau) associated with AD symptoms (Iaccarino et al., 2016).

tACS and Cognitive Functions

tACS has been studied in healthy individuals and results have shown enhanced retention of declarative memories, improved results on N-back tasks, decision making and creativity (Marshall, 2006; Röhner, F. et al., 2018; Hoy, K. E. et al., 2015; Sela, 2012; Lustenberger, 2015).

Changes in crystallized and fluid intelligence in preclinical AD is another thing that can signal progression of the disease. Specifically, preclinical AD has shown to be associated with a fast decline intelligence while crystallized intelligence remains more stable (Harrington, Karra D., et al., 2018). Studies done on fluid intelligence and transcranial oscillatory potentials showed that the gamma band directed towards the prefrontal cortex yielded a 15% improvement when participants did a complex Raven Matrix (neuropsychological test) (Santarnecchi et al., 2013).

On the other hand, tACS studies on individuals with brain disorders such as dementia have also been promising (McDermott, 2018; Martin Prince et al., 2015). Specifically when using tACS to target gamma, since this band is seen throughout memory formations in mice as well as humans (Palop, 2016). Additionally, in healthy individuals as well as AD patients, studies using tACS have resulted in benefits in various cognitive functions like working memory (Röhner, F. et al., 2018; Hoy, K. E. et al. 2015; Santarnecchi, E. et al., 2013; Santarnecchi, E. et

al., 2019). Long term tACS therapy using gamma can modulate PV+ cells through resonance and recover gamma levels (Palop, 2016; McDermott, 2018).

Various other studies have seen effects that lasted even after the tACS treatment was finished (Zaehle, 2010; Kasten, 2016; Antal, 2013). This could be the result of synapses long term potentiation (LTP) or long term depression (LTD). This indicates that long term exposure to the stimulation could encourage long lasting effects.

Research Aims and Hypothesis

Inducing gamma using tACS has the potential to benefit AD patients. The main research goal of this thesis was to assess the feasibility of 10, hour-long sessions of tACS on the working memory and gamma oscillations in AD patients. Based on previous research it is hypothesized that the aforementioned protocol will impact the gamma frequency by increasing its power from the resting EEG recording as well as increase gamma throughout the N-back tasks. As a response to the intervention a benefit in working memory is expected with higher P3B amplitudes in N-back performances. It is to our understanding that this is the first study being done in humans. In order to test this hypothesis the following specific aims were used.

Specific aim 1: Will be to show that gamma oscillations can be induced after 1 hour long sessions of 40Hz tACS stimulation. It is expected to see a change in resting state gamma power post-stimulation. Being able to induce gamma will be useful as research has shown that AD patients show a diminished amount of overall gamma frequency in their

brain, additionally, lower levels of gamma in the hippocampus in mice has been followed by an accumulation of plaques and cognitive disorder.

Specific aim 2: Is to observe improvements in cognition. Using signal averages or ERP's, along with N-Back tasks we can assess working memory and gamma changes. P3, one of the most studied ERP components peaks at ~250-500 originating from a network involved with memory process and attention. Changes in this component are considered an index for decline in cognition, and can be quantified during an N-back task. It is expected to see a reduction in P200 delay throughout stimulations. If the results of the N-back task show improvement then this induction of gamma frequency can potentially result in a benefit to working memory in AD patients. Additionally, other neuropsychological tests will be done to evaluate other cognitive changes such as MMS, ADAS-cog, activities of daily living questionnaire, and the craft story recall test. Using these assessments will be useful to understand the effects of specific aim 1 on cognition.

Chapter II

Materials and Methods

Participants and Study Design

There were a total of 15 participants with an existing diagnosis of mild to moderate AD. The participants had a median age of 76 years, with a mini-Mental State Exam score median of 25, and an ADAS-Cognitive Subscale median of 17. Participants were assigned to three different groups (5 per group), each group received 10 daily sessions from monday-friday of tACS. All participants provided their consent prior to participating in any study procedures. Study followed the international ethical guidelines for biomedical research involving human subjects.

Group 1 received personalized electrode montages that targeted each patient's respective Aβ deposition, group 2 received 10 sessions of bihemispheric tACS that targets the temporal lobes, and group 3 received the same protocol as group 2 with the exception of 20 sessions instead of 10 (Figure 3). Saliva samples used for BDNF and APOe analysis. Aside from neuropsychological assessments (NPS and ADL) conducted before and after treatment, additional comprehensive neuroimaging assessments were done on the participants, including EEG recordings, MRI, PET (for Aβ imaging), TMS with EEG, and saliva sampling (Figure 2).

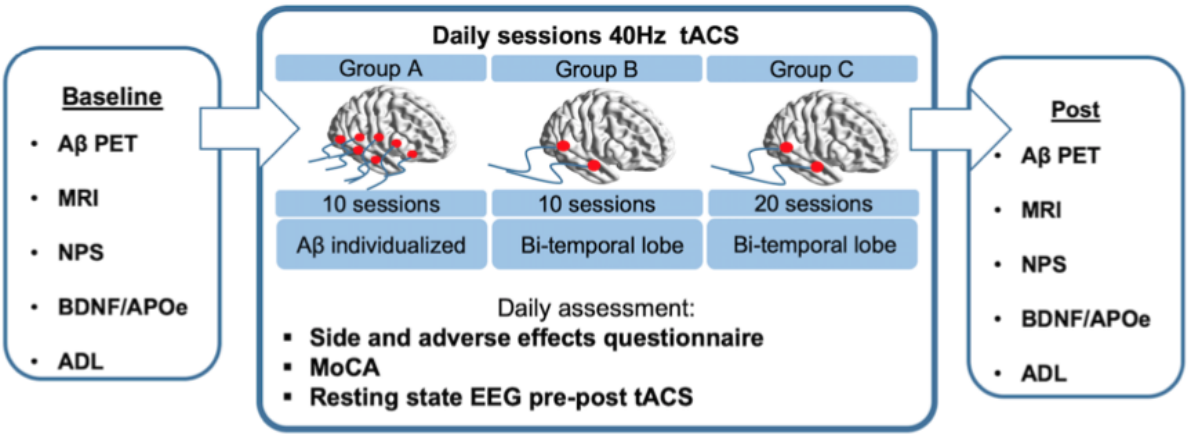

Figure 2. Daily tACS session layout and pre/post intervention assessments.

These assessments include Aβ positron emission tomography (PET), Magnetic resonance imagining (MRI), neuropsychological test for neuropsychiatric symptoms (NPS), saliva samples for BDNF, and activities of daily living (ADL).

Inclusion Criteria

The patient must have been diagnosed with mild to moderate Alzheimer's disease and have acquired a minimal mini mental state examination score of 18 and minimal clinical dementia rating score of 0.5. They also need to have a history of memory impairments and show positive amyloid status in a positron emission tomography (PET) test. If the patient has taken medications for memory loss the dosage must be stabilized for a period of at least 6 weeks. Additionally the participant should have been educated up to at least the 8th grade.

Exclusion Criteria

The participant cannot have any history of the following: intellectual disability, migraines, neurological disorder (other than dementia), intracranial brain lesions, non cortical diseases, major psychiatric conditions, substance abuse, fainting spells, or seizures. Additionally, any participants with neurologic impairment that resulted from surgery or trauma, with recent radiation exposure, with metal implants (such as a pacemaker or cochlear implant), or with uncontrolled medical conditions were excluded. A pregnant woman or breastfeeding mother as well as a participant with any hair style that interferes with the electrode contact was also excluded.

Intervention/treatment tACS

tACS treatment was done at a 40Hz frequency while targeting the areas of greatest tracer uptake from the Aβ PET imaging with an individualized electrode montage to increase the induced current to the targeted region (group 1) or a bi-temporal montage (group 2 and 3) (Figure 3). The daily visits consist of tACS for 60 minutes at a 1 milliamp intensity in each electrode (2mA max per electrode) and a 30 second fade in and fade out period of 30 seconds. There were a total of 144,000 cycles of stimulation every hour.

Each participant received a total of 10 sessions per day lasting a total of 1 hour at 40Hz frequency using a Starstim 32 Stimulator (neuroelectrics Barcelona, Cambridge) with 3.14 cm Ag/AgCl electrodes. The stimulation was delivered at 2 milliamps with 30 second periods of

ramp up followed by ramp down. The participants had electrodes located in a neoprene cap using the 10-20 international EEG system placement, additionally gel was used to optimize signal conductivity (Signa Gel, Parker Laboratories Inc.). During all sessions, 32 electrodes were placed before and after tACS treatment, even though only some were actually being used for stimulations (Figure 1 and 3).

Montage Placement

PET studies have shown that Aβ plaques have greater accumulation in the frontal and temporal regions of the neocortex (Sepulcre J, 2013), for this reason the montages used targeted these two regions. The 5 participants in group 1 had montages derived from individual PET and MRI scans to create their individualized montage (Figure 3). This was done by first using a realistic head model that is used in the stimweaver montage optimization algorithm which takes into account the head model, the target E-field map, the total current, and the highest current power per electrode (Ruffini G, 2014). The target map was seen on the white matter/grey matter interface of the personalized MRI based models ((Ruffini et al., 2018). Each patient had two clusters of Aβ identified. For the bi-hemispheric temporal lobe montage placements, the electrodes P7 - P8 - T7 - T8 were employed with the same phase in every hemisphere.

EGG Recordings

10 minutes before and after the tACS stimulation, EEG was recorded during participant resting-state throughout all interventions. For the recording, the standard 10-20 montage (International System) was used to record the 32-channel EEG (Jasper, 1999). The EEG device used was the Startim 32 (Neuroelectrics from Barcelona, Cambridge) with a right mastoid ground and 500Hz sampling rate. Participants were asked to remain still and relaxed as to restrict as much movement as possible.

Using the Starstim device, electroencephalogram recordings were obtained. The recordings were taken using the same montage as Figure 3, when the participants remained inactive and with opened eyes. The signals were also recorded during a 1-back task 1 week before and after stimulations using the 32 channel montage on Figure 3.

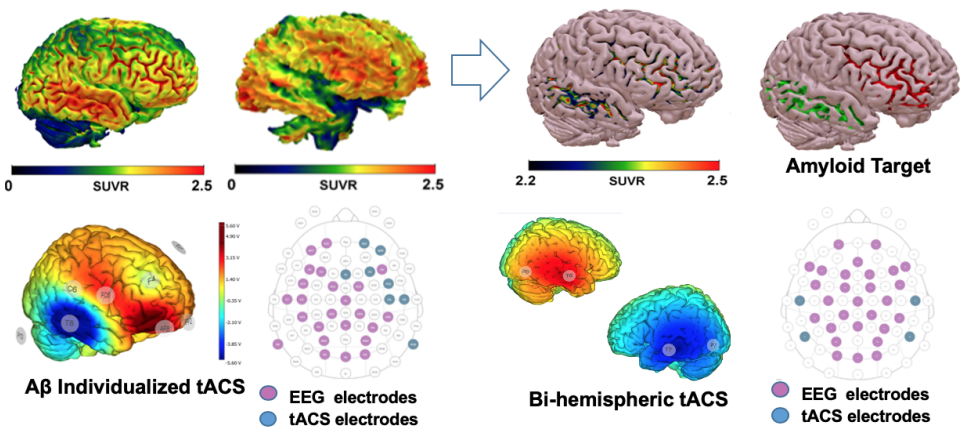

Figure 3. tACS montage.

Group A had an Aβ individualized tACS model that targeted the patients Aβ deposition while groups B and C used the model on the right which targeted the bi-hemispheric temporal lobe. Target maps where defined on CT, Aβ PET and T1-weighted MRI data. Intenities for tACS were set at 2mA −/+1mA, figures above show the standard uptake value ratio (SUVR).

EGG Processing

Brainstorm (Tadel F, 2011) and EEGLAB 14.1 (Delorme A, 2004) were used to develop the EEG data. Recordings were filtered using a low pass filter at 70Hz and high pass cutoff frequency at 1Hz. Notch frequencies were at about 60Hz. Afterwards, using visual inspection, noisy channels where removed and muscular or ocular components were taken and removed using Independent Component Analysis (ICA) from EEGLab 14.1.

For every visit, one minute of clean EEG was extracted pre and post stimulation. The spectral power of each clean signal in each electrode site was measured using Fast Fourier Transform (FTT). Calculations of change in spectral power were made pre and post stimulation, absolute and relative density data was inspected in the different frequency domains. Relative power is considered the percentage of power in a frequency band over the total power of the signal, which has a range of 1-55Hz. The bands included in this range are beta 14-30 Hz, alpha 8-13 Hz, theta 4-7 Hz, delta 1-4 Hz, and gamma 35-55Hz. Within the gamma band there are subsections that include low gamma 35-45 Hz, narrow gamma 38-42 Hz and high gamma 45-55 Hz.

Acute and Delayed Effect

To explore the possibility that the intervention may have had additive or carrying-over effects, an index was created to measure pre and post EEG spectral dynamics. Acute Effect was used to describe the response the day of the stimulation, and Delayed Effect is the index that

describes the possible after effects of the treatment. Acute effect is calculated as follows, $Visit_x$ post-$Visit_x$ pre/ $Visit_n$ pre * 100, and Delayed Effect, $Visit_x$+1 pre –$Visit_x$ post/ $Visit_x$ pre*100. Therefore if the results yield a positive value for the Delayed Effect would mean that the intervention has possible carry-over effects because the participant came back to the following visit with increased power in comparison to their baseline. Adding both the Acute and Delayed effects yields the Total effect, which is the overall power change between the visits.

N-back Task and Event Related Potentials

Past investigations have shown that Alzheimer's patients have impaired working memory function (Baddeley, 1991; Missonnier, 2007), and altered ERP components. Specifically, during an N-back task, mild cognitively impaired patients have a visible delay of P200 latencies when compared to the control (Missonnier, 2007; López Zunini RA, 2016). This delay indicated differences in retrieval and storage stages of working memory. To test the hypothesis that the stimulation protocol can alter the P200 delay, N-back tasks (Kane MJ, 2007; Owen , 2005) were given to the participants pre and post-tACS interventions. Presentation software (Neurobehavioral Systems, Inc., Albany, CA, USA), presented the visual stimuli. The visual stimuli used were pseudo-randomized capital letters from the alphabet from A-J, and were displayed in a 15 inch screen monitor.

To perform this task, a participant was shown a set of letters for which they would need to press a button on their left side if that letter matches that of a previous trial (Figure 4). There were 4 trials allotted to the participants in order to practice and trial the tasks. Participants

practiced with assistance until they received full grasp of the presentation and response keys and could execute the task. After the practice trial period the experimental stimulus is shown. The stimuli provided were randomized (using Presentation Software) and distributed into four blocks, each with 10 targets and 40 distractors. The onset time for the stimulus was 1500ms while the spacing per block was 3000ms.

Changes in event-related potentials (ERPs) and event-related synchronization and desynchronization (ERS and ERD) were analyzed using EEG recordings. One of the participants baseline recording was corrupted and was not included in the analysis.

Event related potential (ERP) calculations where done by separating accurate responses into the following four groups:

1. The responses made to a target before it was presented in the stimulation.
2. The responses made to a distractor before it was presented in the stimulation.
3. The responses made to a target after it was presented in the stimulation.
4. The responses made to a distractor after it was presented in the stimulation.

A correct response to a distractor involves working memory much like a correct response to a target stimulus, therefore the ERP curve of the overall average was calculated as well (Missonnier, 2003 & 2005).

Figure 4. The N-back test set up

This figure shows the different stimuli (G, D, D, S…) that are being used that the EEG is recorded. These recordings were used and averaged to formulate an average ERP waveform per participant.

ERPs and N-back Analysis

ERPs were calculated for all participants as well as the ERP average per participant. Prior to this the ERPs were isolated as to only have the segments including the stimulus. Then, error trials and artifactual trials were removed. A total of 1353 trials of correct responses to distractor and target were used for analysis. Attempts that fell between the -500 to 900ms answering range were analyzed. Electrodes Fz, F3, and F4 were clustered to get a better measure for the P200 component since it has maximum activity over the frontal regions.

Safety and Adverse Events

Recordings of any adverse events pre and post-tACS were gathered throughout the study. An adverse event questionnaire as well as monitoring of cognitive effects were done. To monitor the cognitive effects the Montreal Cognitive Assessment was done, if a participant was to receive a drop of 4 points or higher from the baseline a neurological exam was done (Milani, 2018).

Data and Statistical Analysis

The results for stimulation on Acute, Delayed and Total effects indexes were analyzed using 3 different one-way anova tests. For each band the equation $\Delta Power = \frac{Post_n - Baseline_n}{Baseline_n} * 100$ was used to calculate power changes (ΔPower)(Figure 5) . A positive or negative value for the relative variation of relative powers indicates an increase or decrease in power, respectively. P-Post and P-Pre are the powers before and after the stimulation.

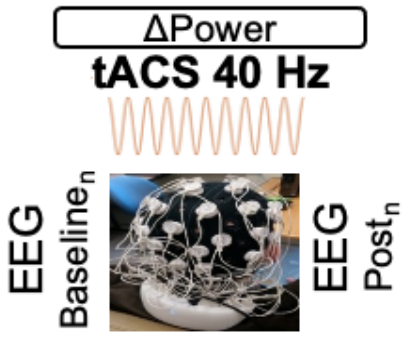

Figure 5. EEG power changes

In order to compare baseline stimulation and post-stimulation variables and N-back ERPs, a dependent sample t-test was done. The Pearson correlation coefficient was used to determine the strength and direction of the correlation between the cognitive variables.

Chapter III

Results

Stimulation Effects

Analysis of the data showed that the increase in gamma power was greater than any of the other bands (such as alpha, beta, and theta), indicating the expected frequency specificity. A one-way ANOVA test was done showing the effect of the stimulation on relative spectral power for each frequency band, yielding an F-value of 5.01, p-value of < .01, and an effect value of η2 =0.32. After further analysis it was evident that gamm power increase was greater in comparison to all other frequencies (Figure 5A). Another one-way ANOVA test was done for the Acute and Delayed effects with Acute yielding an F-value of 2.07, p-value of .06, and an effect value of η2 =.16. Delayed effects yielding an F-value of .44, p-value of .08, and an effect value of η2 =.04.

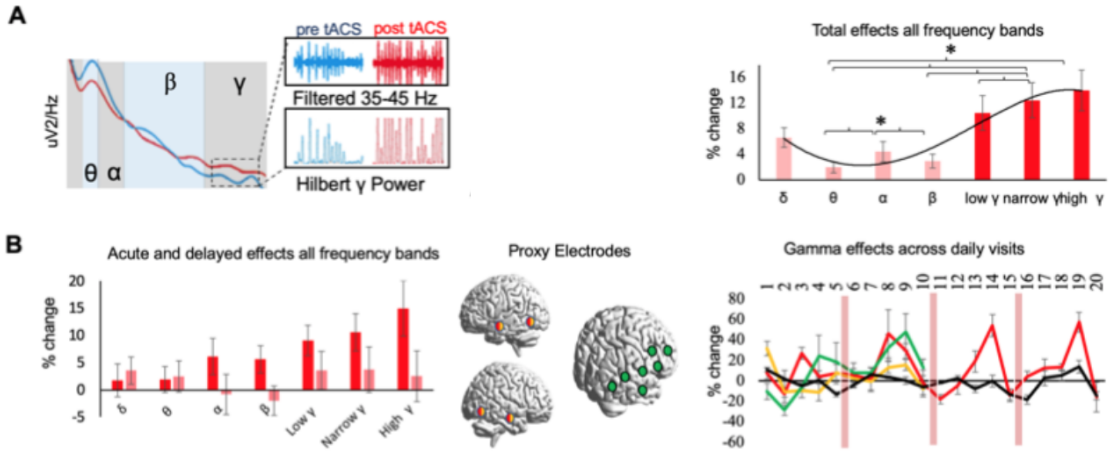

Figure 5. EEG power dynamics and ERPs.

A- The first figure on the left shows the band pass filter at 35-45 Hertz prior to gamma power extraction by Hilbert transformation. The red line indicates after the intervention and the blue

line indicates before the intervention. Here we can see frequent gamma bursts at the T2 position compared to T1 position. The picture on the right hand side shows the ΔPower TOTAL effect with all of the frequency bands and visits. Results of the one way anova test done showed that the effect of the intervention on gamma bands is much more prominent that the effect it had on the lower frequency bands.
B- The picture on the left shows the average in power for ACUTE and DELAYED effects throughout the frequency bands. The picture in the center shows a schematic of the positioning of electrodes for the three different groups. The red and orange sections were applied to groups B and C while the green were for group A. On the right hand side we have the results for the different groups. The green line shows the group with the individualized montages while the orange and red lines are for the groups that received 10 and 20 sessions of bi-hemispheric temporal lobe, respectively. The black line represents a control site. The shaded vertical red rows represent the weekend breaks.

Adverse Effects

The intervention was tolerated why all participants, additionally, all 15 patients fully completed the study. Some patients showed mild adverse events such as hand tingling or strong head pain, but these adverse events were not considered to be related to the intervention by the standing neurologist. Other effects experienced were slight burning, headache, tingling and itching sensations under the electrodes on the scalp (Table 1). Some of these effects, such as the mild headache, were likely due to the mild pressure that the cap applied on the patients head. Aside from what is mentioned above, no other notable effects were noted during the intervention. Lastly, only 9 out of 200 of the scheduled intervention visits were missed by the participants. This data shows the minimal attrition and feasibility of the approach (Table 2).

ID	Group	Sensation			Head Pain			Visual Changes			Nervousness			Scalp Irritation			Neck Pain			Eye Pain			Trouble Concentrating			Hand Tingling		
		F	S	A	F	S	A	F	S	A	F	S	A	F	S	A	F	S	A	F	S	A	F	S	A	F	S	A
1	A	1	1	5	1	1	4	2	2	4	2	1	2	2	1	5	-	-	-	-	-	-	-	-	-	-	-	-
2	A	1	1	5	-	-	-	2	1	4	-	-	-	2	2	5	-	-	-	-	-	-	-	-	-	-	-	-
3	A	2	1	5	-	-	-	2	1	4	-	-	-	2	1	5	1	1	5	2	1	3	-	-	-	-	-	-
4	A	2	1	5	2	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	A	2	1	5	-	-	-	2	1	4	-	-	-	2	1	5	-	-	-	-	-	-	2	1	4	-	-	-
6	B	2	1	5	-	-	-	2	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	4
7	B	1	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	B	1	1	5	2	1	5	1	1	4	-	-	-	1	1	5	-	-	-	-	-	-	-	-	-	-	-	-
9	B	1	2	5	2	1	3	1	1	2	-	-	-	2	1	5	2	1	5	-	-	-	-	-	-	-	-	-
10	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	C	1	1	5	2	3	5	2	1	4	-	-	-	1	1	5	1	1	5	-	-	-	-	-	-	-	-	-
12	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

F= frequency, 1-constant, 2-intermittent; S= severity, 1-mild, 2-moderate, 3-severe, 4-life threatening, 5-fatal;
A= attribution, 1-unrelated, 2-unlikely, 3-possible, 4-probable, 5-definite

Table 1. Information on participants' adverse effects

ID	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	A	✓	✓	✓	✓	✓	✓	✓	x	✓	x										
2	A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										
3	A	✓	✓	✓	✓	✓	✓	✓	✓	✓	x										
4	A	✓	✓	✓	✓	✓	✓	✓	✓	✓	x										
5	A	✓	✓	✓	✓	✓	✓	✓	✓	✓	x										
6	B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										
7	B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										
8	B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										
9	B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										
10	B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓										
11	C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x
13	C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
14	C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x
15	C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x

✓= visit completed; x= visit missed; Group A = 10 sessions of right-hemispheric tACS target each patient's Aβ deposition map; Group B = 10 sessions bi-hemispheric tACS targeting both temporal lobes; Group C = 20 sessions bi-hemispheric tACS targeting both temporal lobes.

Table 2. Daily visits for treatments showing low level of attrition

Sessions Effects

The dose response was further examined for groups B and C, receiving the bi-hemispheric temporal stimulations. There were no apparent differences in the two groups when only looking at the first 10 visits. We did, however, find a difference in group C during the last 10 between the central electrodes (M=-4.91, SD=6.86, $t_{(9)} = -2.28$, p=.04) and temporal lobes (M=10.75, SD=14.04). This information suggests that the stimulation intervention can have a cumulative effect as it sustains an increase in gamma. This information is in accordance with other studies done with similar forms of brain stimulations (Winker C, 2020; D'Agata F, 2016). The data also shows a peak trend in gamma towards the middle of the week (Figure 5B).

Montage Effects

The ACUTE relative power change in gamma for bi-temporal and mono-hemispheric spatial montage positions were observed. The mono hemispheric positions were common of those used for group A with electrodes F2, FC2, FC6, F6 and CP6. While the bi-temporal positions are used in groups B and C with a P8, T8, P7 and T7 montage. Central electrodes were used as control regions and had electrodes CP1, CP2 and Pz. The data showed a difference between the mono-hemispheric electrode and the control as well as with the bi-temporal electrodes and the control, indicating a greater increase in gamma in areas undergoing stimulation underneath the electrodes.

ERPs Effects

N-back memory tasks were completed by the participants before and after intervention. Analysis of the event related potentials before and after stimulation were conducted using a paired t-test. After correcting for multiple comparisons, the results showed a reduction in the P200 latency post-stimulation with a p-value < .05. This is a possible indication of increased storage phase and working memory retrieval. Figure 6 shows a grey rectangular area that highlights the significant latency between pre and post stimulation indicated as the blue and red light, respectively.

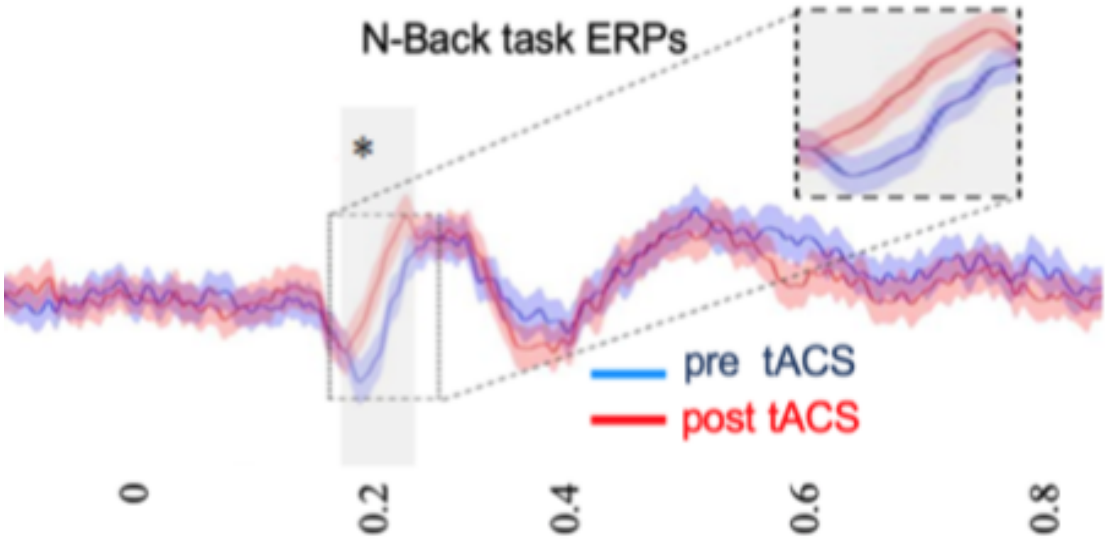

Figure 6. tACS effects on working memory ability. The light blue lines represent pre tacs intervention while light red lines show post tacs intervention. The shaded gray area shows a significant change between pre and post tacs interventions. Stimulation seems to have effectively modified information processing during the working memory task, as demonstrated by the earlier onset of evoked EEG potential related to stimuli processing. A delay in such potential has been documented in both AD and MCI patients, suggesting tACS as a potential tool to restore physiological cognitive processing mechanisms in dementia.

Cognition Effects (Global cognition, ADL, declarative memory)

Since the intervention was short-lived, large changes in cognition were not expected. Cognitive assessments were conducted mainly for the purpose of assessing the feasibility and safety of the stimulation protocol. These expectations were met, however, results also showed that some participants had improvements in language tasks and declarative memory and that there may be an association between greater gamma synchrony and language and memory.

Patient's declarative memory changes were analyzed using the craft story recall test (CSR). These results showed that the majority of participants bettered their language and declarative memories. The data showed a significant difference between the baseline (M=3.87, SD=3.36) and post (M=4.93, SD=4.30) with effect size Cohen d = 0.3 (Figure 7). 75% of the participants reported having improved word finding ability and better episodic memory in their daily activities throughout the intervention. These findings were confirmed by feedback provided from the participants' caregivers.

Additional assessments of global cognition and activities of daily living (ADL) remained stable throughout the intervention. Global cognition remained the same, as per the ADAS-Cog neuropsychological assessment (baseline M=18.27, SD=7.68 and post-tACS M=18.11, SD=7.69). ADL assessments were done using questionnaires involving normal everyday activities such as dressing, showering, eating et cetera (Figure 7).

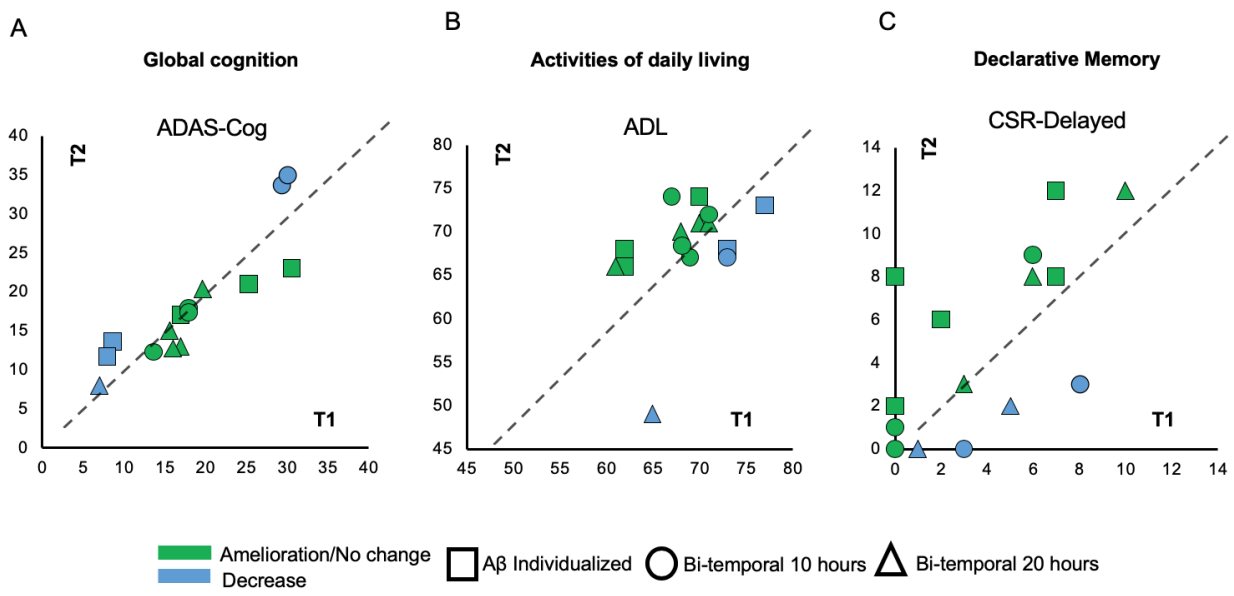

Figure 7. Stimulation effects in global cognition, activities of daily living, and declarative memory results. The X axis T1= pre tACS intervention, y axis T2= post intervention. ADAS-Cog higher scores indicate greater cognitive impairment. CSR-Delayed paraphrases receive a point for every response provided that correctly captures the events of the stories.

Using Pearson correlation, a positive association between greater gamma synchrony and improved performance in language and memory were detected. There was an improvement in CSR-delayed with increases in gamma connectivity (CSR-Delayed $r_{(15)} = 0.6$, $p = .024$), and even greater correlation observed for b-hemispheric temporal lobe stimulation (Figure 8).

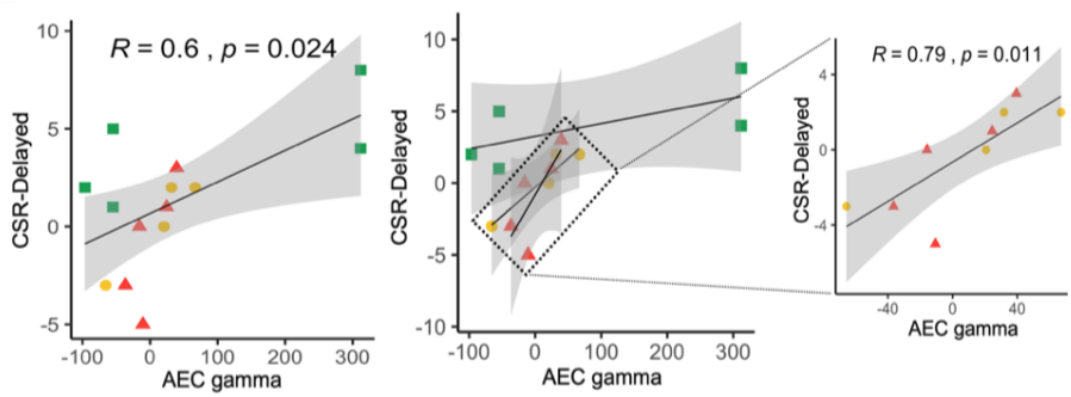

Figure 8. Graph of pearson correlation calculations between gamma power amplitude envelope correlation changes (AEC) and changes in declarative memory using CSR-Delayed.

Chapter IV

Discussion

Significance of Results

To our knowledge, there has not been any other study investigating how a 40 Hz intervention of transcranial alternating current affects patients with Alzheimer's disease. This study is a first in-human translational trial and supports the recent study of the effects of gamma induction in mouse models of AD (Adaikkan C; 2020; Iaccarino, 2016; Hipp, 2012). The overall results provided evidence to the feasibility of intervention and the ability to induce gamma oscillations using tACS immediately after stimulations and that low gamma can be incremented greatly in temporal and frontal areas. AD patients were taken through an intervention protocol with the objective of inducing gamma oscillations after 1 hour long sessions of 40HZ tACS stimulation. The results indicate that when compared to the other bands, gamma had an increase in power. Additionally, results showed that there were the greatest effects of the stimulation were observed in the areas of electrode placement, showing efficacy and specificity of the intended approach. Another observation made was that as more stimulations sessions were done, the greater the gamma oscillation impact, suggesting that the effects are cumulative and dose-dependent. The data analyzing the changes in cognition also proved promising as they showed improvements in working memory, activities of daily living, declarative memory, and language.

Feasibility and First Time in Humans AD

Previous studies have explored and provided evidence for the feasibility of applying tACS to healthy humans as well as inducing gamma in mice with AD. Specifically, the study done by Iaccarino et al observed changes in gamma oscillations using optogenetics, the findings showed that the gamma oscillations recruited glial and neuronal responses in the brain which results in AD pathology attenuation. Additionally, a human study using 40HZ (Antal A, 2017) used sessions that only lasted 10-20 minutes each. This is the first human trial exploring the effects of inducing gamma on patients with Alzheimer's Disease patients and that shows the feasibility of 1 hour-long daily stimulation sessions of 40Hz tACS.

The study resulted in a low level of attrition and adverse effects. Any adverse effect related to the intervention where very minor and can be dealt with in future studies with simple alterations such as by adding conductive gel or making a more comfortable environment for the participant. Considering the vulnerable clinical conditions of the AD patients, it is noteworthy that no serious adverse effects were recorded.

Increasing Gamma For the First Time in AD

Patients with AD usually have a decrease in higher frequency oscillations such as those in the gamma band (Nimmrich, 2015). Recent studies have gone off to better understand this deficiency and what the role in gamma is in Alzheimer's Disease. Studies found that this deficiency in gamma had some sort of relation to the aggregation of Aβ, and once the deficiency

was targeted with optogenetic and multisensory techniques resulted in positive effects towards AD pathology (Martorell , 2019; Sohal, 2009;Cardin, 2009; Adaikkan C, 2019; Adaikkan C, 2020). Our study is the first to show that tACS can result in a substantial increase in gamma oscillations for AD patients. This information is important because a typical sign of AD is a general EEG slowing (Stomrud E, 2010).

Cumulative Effects

Throughout the multiple daily sessions of stimulation, a cumulative effect was observed. Results showed that as the sessions progressed, the effects became greater. This may be as a result of Hebbian plasticity, where synaptic efficacy results from the repeated stimulation between the pre and postsynaptic cells (Koch G, 2013; Revill, 2020; Huang, 2017). Further analysis of the data shows an increased effect halfway through the weekly intervention, after which the data shows a drop. This could be the result of habituation, in which the response to the given stimulus decreases due to its prolonged presentation. After the drop, the participants spent the weekends, two days, free of tACS possibly allowing for a period of dishabituation. When a new week began the additive effects continued again.

Cognitive Effects

Neuropsychological assessments were done to assess the different cognitive domains such as memory, language, attention, executive, and spatial functions. After the stimulation,

results showed some improvement in declarative and working memory. The improvement in declarative memory is very noteworthy because this is the type of memory most prominent in early stages of the disease.

Working memory was explored using the N-back tasks, the results showed a reduced P200 latency period post-stimulation. This is important because mild cognitively impaired patients have visible P200 latencies when compared to controls (Missonnier, 2007; López Zunini, 2016). This delay indicates differences in retrieval and storage stages of working memory. These results indicate that the induction of gamma using the stimulation protocol in this study improved the working memory processing in the participants.

Further analysis was done to confirm that these improvements in memory were in fact related to the induction of gamma. This was done by taking a close look at the relationship between cognition and EEG resting power for each frequency. Results showed a relationship with gamma band and declarative memory only. The findings of this study are in congruence with previous studies done in healthy subjects undergoing bi-hemispheric temporal and frontal tACS at 40Hz. These results support the idea that inducing gamma oscillations can contribute to better cognitive processing and that this is an effective approach to bettering the behavioral and pathological symptoms of AD (Palop, 2016; Adaikkan C., 2020).

Qualitative reports from family members were very positive as well. The wife and sister of one participant mentioned that the patient has been saying how things are less fuzzy than they used to be, that he is more engaging in conversations and that his speech has improved. Another patient mentioned that it is easier to remember things such as where they placed the keys and

when they need to get new tires. Some patients' study partners claimed the subject has reversed back to where they were with their disease about 2 years ago. Additionally, other family members of patients have witnessed a global impression of change, as the entire family and friend group think the patient is like his "old-self". Other study partners have witnessed small things such as having the patient flip the calendar to the correct month to mark when they had to take their next medication, an act that had recently become uncommon for the patient to carry out on their own.

Altogether, these findings of gamma induction with minimal levels of attrition and no major side effects indicate that inducing gamma using 40Hz tACS can serve as a promising therapy for Alzheimer's patients. The results, although short (only 2-4 weeks) and introductory, show signs of a potential effect of inducing gamma on cognitive processes. Collectively these findings provide additional knowledge to the ever-growing non-invasive brain stimulation field.

Study Limitations and Future Directions

There were various limitations that could be improved for further studies, one of which is the sample size used for this feasibility study which limits the strength of the results. Additionally, not setting control conditions for this study is an important limitation. Further research warrants a double blind design to verify the results are truly caused by the stimulation and to cross out any confounding variables or placebo effects. Debatably the most important

future study would be that which assumes the feasibility and further explores the reduction in Aβ or disease symptomatic reduction in a controlled setting.

Additionally, this form of technology also has its limitations, including the area in which can be stimulated, the scope or degree of penetration, and the control we have over the area we are working on. Therefore the main limitations would be the lack of control that the researcher has over which area is being stimulated and the systematic monitoring of the effects and how long they will last along with what lingering effects they might have post treatment (Brunoni et al., 2012).

Fine tuning the diagnostic approach for AD will provide greater clarity for the best treatment. Plasma tau/amyloid 40/42 ratio has the potential to predict brain tau deposition and neurodegeneration in AD. Future studies can explore the idea that plasma tau/Aβ ratios serve as a biomarker of brain tau deposition and neurodegeneration for AD. Currently, blood based biomarkers available for AD are PET and cerebrospinal fluid. Unfortunately these markers are expensive or invasive. The human blood is a great source for proteasomes, making plasma a promising new direction for diagnosis. AB1-42:AB1-40 ratio has potential to be used as a diagnostic marker that shows significant correlations between plasma and CSF levels and ratio. Ab40 in cerebrospinal fluid has been found to be many times more than Ab42. The AB1-42:AB1-40 ratio was found to be lower in individuals with AD (Hampel et al., 2018). Additionally, researchers understand that there are Aβ aggregates in skin and subcutaneous tissue, while there is also more Ab40 in the body (peripheral) and Ab42 is considered to be more dominant in the central brain.

The studies duration is also limiting, although the results were encouraging, the observations could have been representative of increased processing speed and basic attention levels as a result of practicing. Further studies are warranted with controlled settings as well as longer task durations in order to get a strong ERP calculation. Additionally, it could be useful to further explore the effects on earlier stages of the disease.

AD patients generally find difficulty in sustaining attention, this presents a challenge for event related activities. Future studies can adapt eye tracking methods used with infants which present a similar challenge. Additionally, research has found an association with deprivation of sleep and Aβ burden (Mander, 2016; Shokri-kojori, E. et al., 2018) as well as an association with sleep deterioration and worsening of pathophysiology and clinical symptoms of the disease (Romanella, 2020). Aside from exploring the effects of sleep quality on treatment response it might be worth exploring the fact that gamma oscillations are present throughout slow wave sleep (Valderrama, M. et al., 2012). It is possible that during this stage there is a greater response when the brain receives stimulation.

It is important to better clarify the mechanisms of the stimulation in humans as well as the application to the clinical population. Additionally, research by NASA has shown that galactic cosmic radiation has been shown to lead to cognitive impairments and an increased Aβ Plaque accumulation in mice, suggesting exposure to deep space as a potential accelerated model of AD . Future directions have begun to investigate how tACS can minimize the deteriorating effects (such as the accumulation of Aβ in mice models) of cosmic radiation and microgravity (Sprugnoli, 2020).

Although transcranial current stimulation both direct and alternate are a very popular topic for non-invasive brain stimulation investigations, there aren't many studies on the neurobiological mechanism associated with tACS. We need to better understand the impact of parameters of stimulation, whether short periods of stimulation don't produce many effects or produce beneficial effects and whether longer simulations produce better effects or have the ability to produce harmful effects after a certain time and intensity (Brunoni et al., 2012).

There also needs to be further study of the interactions between this noninvasive brain stimulation and pharmacological treatments especially considering Alzheimer's patients that might be taking various medications simultaneously. The long-term effects of transcranial current stimulation have not yet been fully addressed. There is also a need to further understand each functional role of the cortical areas as well as establish safety guidelines.

In order to make longer tACS treatments feasible (for over 3 months) it is warranted to consider a treatment plan in which the patient can receive stimulation at home in a controlled and previously adjusted setting. Possibly if further research on gamma inducing music is done, it can be considered an additional at home simple treatment.

Other populations of patients can also benefit and build from this study since the dysfunction of GABAergic activity and interneuron pathology partakes in the pathophysiological process of several neuro and psychiatric disorders (gamma deficits) such as schizophrenia, autism, and traumatic brain injury. Additionally, exploring the relationship between level of education and stimulation response from tACS using N-back tasks would be an interesting study

that can further educate us on neuroplasticity. Other topics for future studies include closed loop stimulation using EEG-tACS and improved target identification via PET.

Conclusion

In this thesis we researched the cognitive, clinical and neurophysiological impacts of using tACS to induce gamma in AD. Results showed that gamma oscillations were induced post-stimulation. Working memory was also explored throughout the stimulation protocols during working memory N-back tasks and the results suggest benefit to cognitive processes. To our knowledge this has been the first study in humans using tACS at 40Hz to induce gamma oscillations through a 10 day period. Considering the study results, additional research under controlled conditions and greater sample size receiving 40Hz stimulations warrants exploration as well as further researching the acute versus long term impacts in Aβ and p-tau. Moreover, if this protocol serves as a way to reduce the amyloid plaques and p-tau accumulation in Alzeihmers patients then this provides a possibility for the patients to have a noninvasive and non-pharmacological solution to managing their disease. Overall our findings provide mechanistic insight, knowledge about feasibility, and characterized dose response to further inform future clinical trials inducing gamma using tACS on AD. It is no wonder that tACS is a rapidly growing field as it shows much promise for brain modulation.

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