Ollscoil na hÉireann, Corcaigh

National University of Ireland, Cork

Investigating the Effects of Oscillating Sounds on the Memory of Older Populations

Volume 1 of 1

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For the Degree of

Master of Research

University College Cork

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2019

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Declaration

I declare that this thesis is my own, original work and has not been submitted, in whole or in part, for any other degree. The work was carried out under the supervision of Prof. Cora O'Neill, Dr. Jason Chan and Dr. Suzanne Timmons between October 2018 and September 2019, in the School of Applied Psychology and School of Biochemistry and Cell Biology, University College Cork, Ireland.

Hadley Pfalzgraf

Signed: Hadley Pfalzgraf
October 2019

Acknowledgements

This project has evolved a lot since my first skype meeting with Cora O'Neill over a year ago. I had seen her profile on UCC's website and appreciated how she used her expertise on Alzheimer's to effect social change and advocate for patients and caregivers. She soon put me into contact with Suzanne Timmons and Jason Chan who each contributed their unique skillset to the project as well.

Thank you to Suzanne for helping with ethical approval and opening doors in the clinical community. Thank you to Jason for your endless patience and infectious excitement about neuroscience. I often entered our meetings confused and disheartened by the results of data analyses, but I always left reinvigorated and more excited to figure out what was going on from our conversations. And thank you to Cora for being willing to take a chance on me and keeping me and everyone else on track throughout this entire process.

Thank you to Sarah O'Donovan and Rachel Furlong for the moral support and endless laughs (special thank you to Rachel for picking this up from the printers and turning it in!). Thank you to the Mitchell Scholarship and the US-Ireland Alliance for making this master's degree possible. Also, thank you to Ireland for the hospitality and such a wonderful year. And a huge, heartfelt thank you to all of the participants who donated their time and made working on this study such a joy.

Abstract

Introduction: Memory functions are associated with various oscillations of electrical activity in the brain, and disruptions of those rhythms can be observed in many neurological syndromes, including Alzheimer's Disease. Oscillations at the theta (3-7 Hz) frequency, in particular, are thought to play an important role in memory. The peak frequency and amplitude of the theta frequency are modulated by age and could be a useful target to combat the cognitive decline commonly associated with aging. Previous studies have induced theta oscillations using transcranial Direct Current Stimulation (tDCS) and repetitive Transcranial Magnetic Stimulation (rTMS). We hypothesized that oscillatory auditory stimulation could similarly entrain theta rhythms in a less invasive, more cost-efficient manner. Preliminary data collected from young adults indicated a positive relationship between theta entrainment and improved short-term visuospatial memory.

Methods: We recorded the neural activity, using electroencephalography (EEG), from participants aged over 50 years while they completed a spatial memory task. Three groups of participants with varying memory ability were recruited with no memory complaints (n = 15), mild memory complaints (n = 16), and moderate memory complaints (n = 16). During the first part of the task, participants listened to amplitude modulated noise at 3, 4, 5, 6, and 7 Hz to determine which frequency within the theta band induced the strongest increase in theta power with respect to a pure noise control. Next, participants learned the locations of 30 objects via a spatial memory task administered on a computer. Each object was consistently paired with one of three types of pink noise—constant noise, individualized theta frequency-modulated noise (3, 4, 5, 6, or 7Hz), and 15-Hz-modulated noise (beta).

Results: Individuals who identified as having moderate memory complaints had significantly lower MoCA scores than individuals with no memory complaints and preferred lower theta frequencies. Individuals with moderate memory complaints also exhibited lower memory

scores in the spatial memory task. Male participants (n = 17) on average displayed higher memory scores compared to female participants (n = 30), despite similar age and memory group distributions. Sounds at an individual's preferred theta frequency led to an increase in theta activity in the brain compared to pure pink noise and beta sounds. Additionally, there was a main effect of memory complaint group on neural activity in the theta and alpha bands. **Conclusions:** Subjective memory complaints may be an accurate proxy for the beginnings of age-related neurological changes reflected in EEG activity. More research in this area could contribute to novel diagnostic techniques and therapies for Alzheimer's Disease. Auditory stimulation could be an easy, non-invasive method to promote beneficial neural activity in aging populations.

Chapter 1: Introduction

Alzheimer's Disease (AD) is an escalating public health crisis, impacting not only the elderly, but caregivers, family members, healthcare systems, and economies.[1] Fundamentally, AD is a disease of the synapse associated with progressive and irreversible memory impairment as well as several other deficits in cognition and executive function.[2–4] The neuropathology of AD is characterized by synaptic loss with excessive accumulation of two protein deposits, namely amyloid beta plaques and tau-containing neurofibrillary tangles (NFTs).[5–7] Unsurprisingly, much research has focused on these molecular pathologies, but no useful pharmaceutical treatment has emerged.

Thus far, most work on AD has been divided along molecular and systems level neuroscience with minimal communication and overlap. The disease's complexity requires the bridging of knowledge from both of these fields. Age is by far the greatest risk factor for AD.[8,9] Notably, molecular and electrophysiological changes are evident in adult brains long before symptoms of AD manifest .[10–16] Therefore, it is important to investigate the neural activity of middle-aged individuals to identify changes associated with normal ageing, in addition to those that may indicate future pathology and risk for the development of AD. The objective of this introduction is to examine how molecular changes associated with 'normal' ageing, as well as changes associated with AD, influence the circuitry of neural oscillations and impact memory.

1.1 What are Neural Oscillations?

Neural oscillations are electrical rhythms produced in the brain that reflect the synchronized activity of many neurons (Figure 1). These rhythmic fluctuations allow neurons to alternate between periods of increased excitability (more likely to 'fire') and decreased excitability (less likely to 'fire').[17] These rhythms facilitate communication within and across brain regions.[18,19] An individual neuron may naturally oscillate at a specific frequency, but it is also

under the influence of the rhythmic population dynamics.[19–21] The increased complexity and opportunities for coordination that neural oscillations provide in the brain has led researchers to equate oscillations with aspects of higher level cognition, including memory.[18,22–24] Traditionally, neural oscillations are broadly grouped into bands according to frequency: 1-3 Hz (delta), 4-8 Hz (theta), 8-12 Hz (alpha), 13-30 Hz (beta), >30 Hz (gamma). Although each band has been linked with different functions, ageing is most commonly associated with an overall slowing of neural activity.[25]

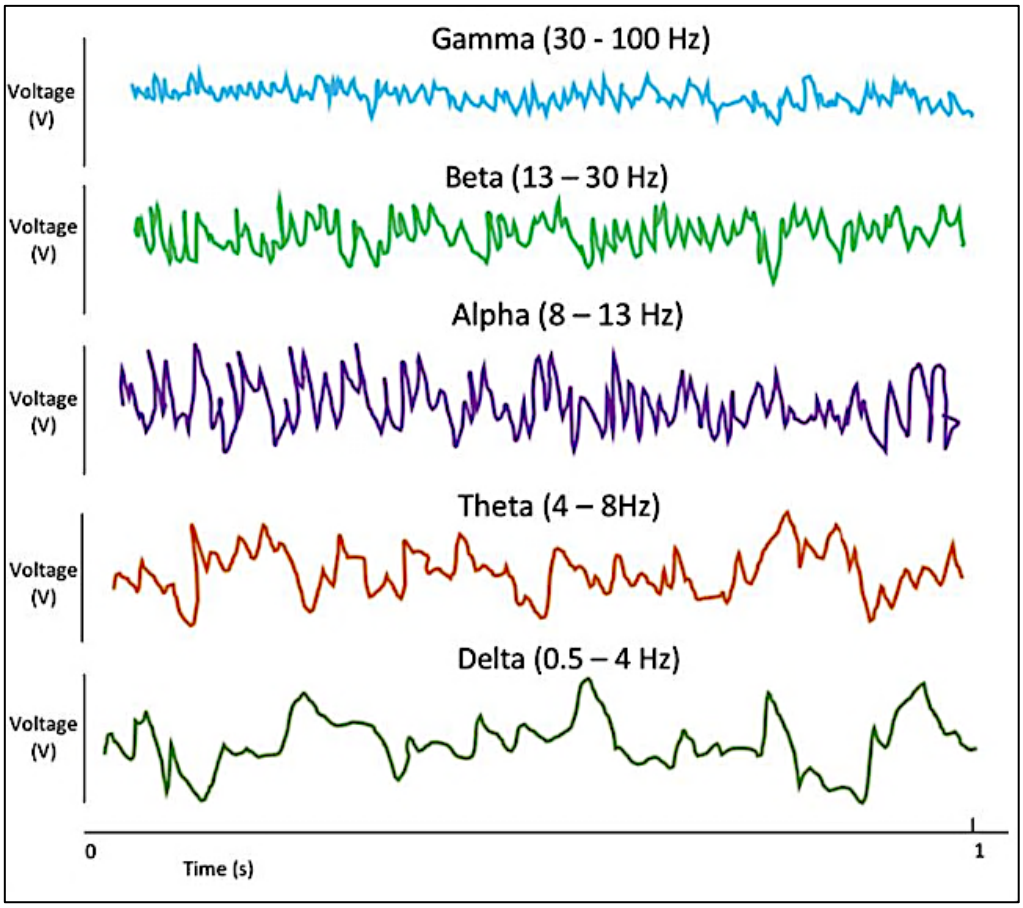

Figure 1.1: Image from Barry et al. 2018 depicting how oscillations in the brain are classified according to frequency into bands.[26]

1.2 Importance of Theta Oscillations in Memory Function

The theta frequency band was originally linked with memory because it is a prominent network pattern in the hippocampus—a structure made famous by patient H.M. who was unable to form new episodic memories after the removal of his hippocampus.[27,28] Memory is

grouped broadly under two categories: short-term memory/working memory which typically lasts less than a minute, and long-term memory which lasts much longer. Long-term memories can be implicit (unconscious) or explicit (conscious).[29] Implicit memory encompasses procedural memory that allows you to remember how to tie your shoes without thinking about it—this type of memory was unaffected in HM and is largely unaffected in aging as well.[30] On the other hand, both types of explicit memory, termed episodic and semantic, can be impacted by aging and AD. Episodic memory encompasses memory for life events and experiences (including spatial memory), and semantic memory encompasses memory for facts and concepts.[29]

Although theta activity may have diverse roles in different types of memory, this study will focus on the role of theta activity in episodic, semantic, and specifically spatial memory. Additionally, there is extensive literature on the importance of theta activity during spatial navigation of rodents in relation to hippocampal place cells, but this will not be explored here in favor of focusing on human studies.[31,32] Originally, theta activity directly from the hippocampus could only be studied in small mammals, but intracranial recordings from epileptic patients have provided an opportunity to more thoroughly study human hippocampal theta activity.[33,34] By utilizing patients with intracranial electrodes it was confirmed that, similar to rodents, theta oscillations can be seen in the human hippocampus during a virtual movement and search task.[35] Hippocampal and neocortical theta were also strongly correlated, giving credibility to the use of scalp electroencephalogram (EEG) recordings to detect theta activity.[35,36]

To further provide a link between hippocampal theta and memory, pre-stimulus theta activity in the hippocampus was associated with successful memory encoding of words.[37] Similarly, increased theta activity during encoding was linked to successful recall of lists of common nouns.[38] Intracranial theta and high-frequency activity were also linked with

spontaneous verbal recall of episodic memories.[39] All of these intracranial studies lend credibility to the importance of theta activity in memory. Furthermore, although theta activity is generated in the hippocampus, it can also be observed over distributed brain networks and areas. Several of the intracranial studies also detail an interaction between theta activity and high frequency activity. This coupling between theta and gamma activity is thought to be important for the communication across brain areas that is integral to cognitive processing.[40] This coupling is also vulnerable to ageing and is significantly impaired in several neurological disorders including AD.[41–43]

On the molecular level, Law and Leung found that synaptic plasticity and spike excitability in the Cornu Ammonis-1 (CA1) are modulated by the phase of the theta cycle in the hippocampus.[44] Increases in theta and gamma activity correlate with long-term potentiation (LTP), or the strengthening of excitatory synapses associated with learning and memory.[45,46] This activity can be recorded electrophysiologically and indicates inputs that occur with the peak of the theta cycle and reach their postsynaptic target within the gamma time window (10-30 ms) are selectively potentiated.[47] Some argue that theta synchronization is the 'glue' that binds the multiple components of an episodic memory together into a coherent episode.[48] Depending on which phase of the theta cycle signals arrive at, it could result in increased excitability, LTP, or long term depression (LTD), the antithesis of LTP.[44] Therefore, theta oscillations play a role in selecting which information gets potentiated as well as binding elements of memories together.

1.3 Hippocampal Theta Circuitry

To understand how ageing and memory function impact the generation of theta oscillations, a brief overview of theta circuitry will be given. Although theta is one of the most widely studied brain rhythms, a comprehensive understanding of its generation and function has not been reached, therefore, we will be operating off of some of the more substantiated theories.

Theta generation is dependent on the cortical-hippocampal circuit (Figure 1.2). In this circuit, rhythmic theta activity from the entorhinal cortex (EC) drives the circuit via the perforant path targeting the dentate gyrus.[49] The granule cells of the dentate gyrus act as a filter, meaning only signals strong enough to activate mossy synapses (large synapses with multiple neurotransmitter release sites) will excite Cornu Ammonis-3 (CA3) pyramidal neurons.[17] The recurrent axons of pyramidal cells in CA3 amplify the activity from the dentate gyrus and synapse on excitatory Schaeffer collateral axons in CA1.[50] To complete the circuit, excitatory neurons from CA1 project back to the EC. Additionally, long-range interneurons provide inhibitory input and synchronize activity between different regions of the hippocampus and cortex.[17]

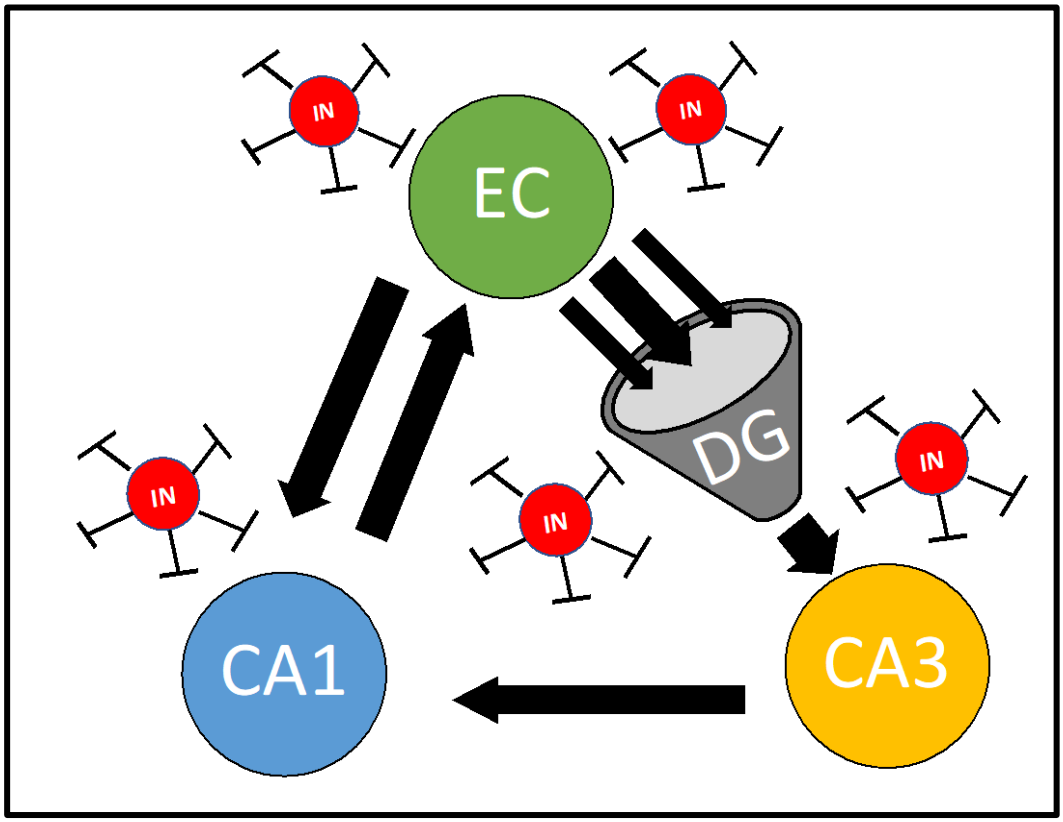

Figure 1.2: Cortico - hippocampal circuit diagram showing the interaction between the entorhinal cortex (EC), Dentate Gyrus (DG), Cornu ammonis 3 (CA3), Cornu ammonis 1 (CA1), and Inhibitory Interneurons (IN).

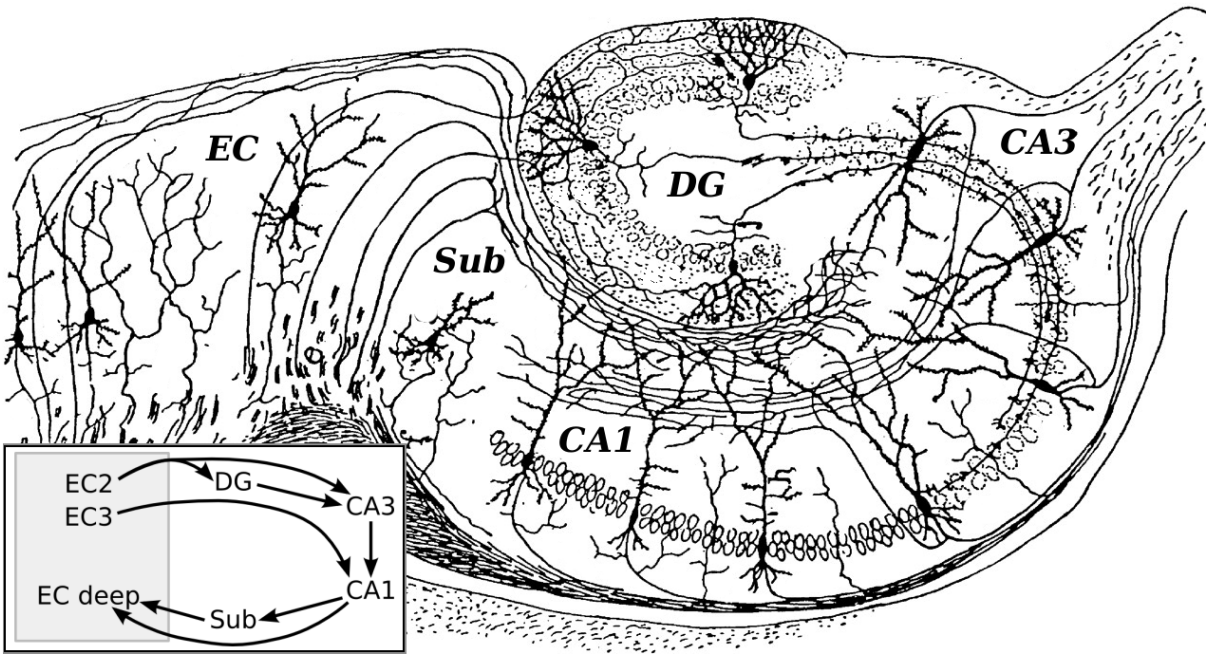

Figure 1.2: Drawing of the rat hippocampus by Ramon y Cajal 1911 for reference in relation to the theta circuitry described above. From: Santiago Ramón y Cajal. (1911) Histologie du Système nerveux de l'Homme et des Vertébrés, Paris: A. Maloine.

1.4 Relevance of theta circuitry to Alzheimer's Disease and ageing

As we age, just as we lose muscle mass, we lose brain mass. The brain loses synaptic connections and myelination, which disrupt neural oscillations and communication in the brain.[51] Theta circuitry is particularly vulnerable to the ageing process. The hippocampus and EC are among the first areas to be affected by ageing and are severely impacted by AD pathology.[52–54]

1.4.1 Overview of Alzheimer's: the major memory disorder of ageing

AD is the most common neurodegenerative disease of ageing. The disease slowly and selectively destroys brain circuits that control memory and higher cognitive function. AD is a chronic disease and is not an accelerated form of ageing.[5] The cause of AD is unknown, and the vast majority of AD (98% of cases) are known as "sporadic".[55] However, age is by far the greatest risk factor.[56] There are genes that can increase an individual's risk for developing sporadic AD, including inheritance of epsilon e4 alleles of the Apolipoprotein E (*APOE*) gene.[57,58] Rarer earlier age-of-onset AD cases, accounting for approximately 1-2% of all AD

cases, are due to autosomal dominant inheritance of mutations in APP (amyloid precursor protein) and Presenilin genes.[59–61]

1.4.2 Alzheimer's: overview of pathology

The most notable anatomical characteristic of AD is the extensive pathology that builds up in the brain as the disease advances. As previously mentioned, the pathology is primarily composed of deposits of two soluble proteins that, for a myriad of poorly understood reasons, begin to aggregate into deposits known as Aβ peptides (made of amyloid-beta proteins) and neurofibrillary tangles (made of tau proteins).[3,5,62,63] These aggregates are associated with an inflammatory response perpetuated by microglia and astrocytes all of which are associated with selective synaptic and neural circuit degeneration.[64–67] It is important to note that both Aβ and tau have normal functions in the brain and when they start to build up there is normally no cognitive impairment.[6] It is when these plaques and tangles have accumulated for longer time periods, anything ranging from 5 to 20 years, that cognitive symptoms may start to manifest.[68] There is thus a continuum of neuropathology and cognitive deficits, from completely healthy to pervasive disease.[5] However, there are many cases that lie in the middle of this spectrum that can be more difficult to categorize.[63,69] As technology continues to advance, hopefully this distinction will become more definitive in coming years.

We know that as the level of Aβ and tau increase significantly an individual may develop mild cognitive impairment (MCI), which may progress into AD dementia (Figure 1.4). As seen in Figure 1.4, levels of Aβ seem to plateau before cognitive symptoms manifest, with significant increases in tau in combination with high levels of Aβ more strongly correlating with neurodegeneration and cognitive decline.[68] Notably, the areas of the brain that Aβ and tau build-up overlap definitively with theta circuitry which will be explored

further below. Furthermore, alterations in theta circuitry have been described in AD and functionally linked to both Aβ, tau, and neuroinflammation.

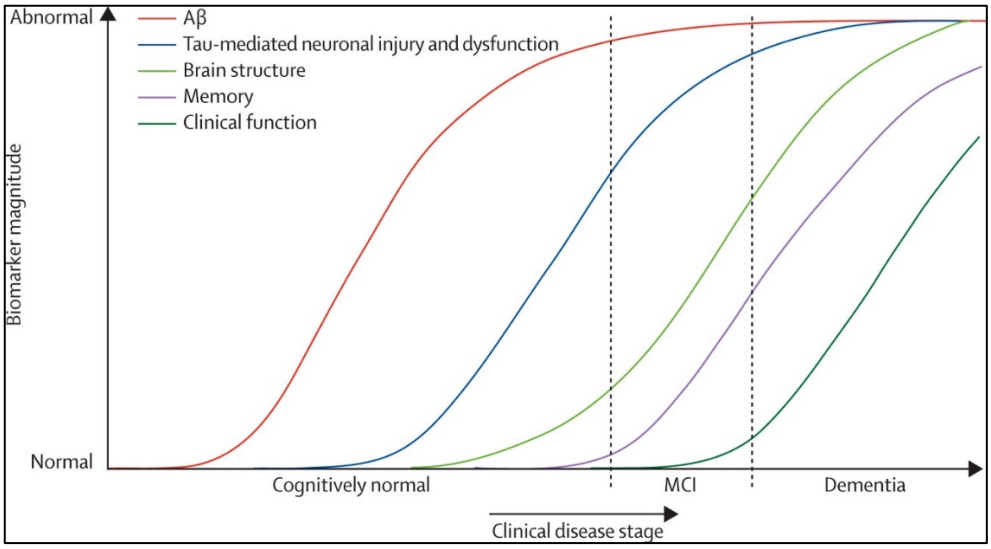

Figure 1.4: Image from Jack et al. 2013 depicting how amyloid beta plateaus before cognitive symptoms manifest, but its combination with high levels of tau correspond to neurodegeneration and cognitive decline.[68]

1.4.3 Alzheimer's pathology and theta circuitry

Remarkably, theta circuitry maps extremely closely with changes seen in aging brains associated with Alzheimer's Disease (Figure 1.5). In the 1990s, the neuropathologists Braak and Braak developed a six-step staging system of human brain pathology (Stage I-VI), which differentiated between pre-symptomatic (Stage I-II), mild cognitive impairment (Stage III-IV), and clinically diagnosed AD (Stage V-VI).[52] Braak staging was based on thorough examination of thousands of post-mortem brains.[52,70] The staging outlines the progressive accumulation of tau in NFTs, plaques, and neurodegeneration, but particular focus is devoted to tau in NFTs.[70] By piecing together information from many brains of different ages, cognitive ability, and AD diagnoses, Braak concluded that NFT changes developed

according to similar patterns across brains as memory loss emerged with ageing and advanced to AD.

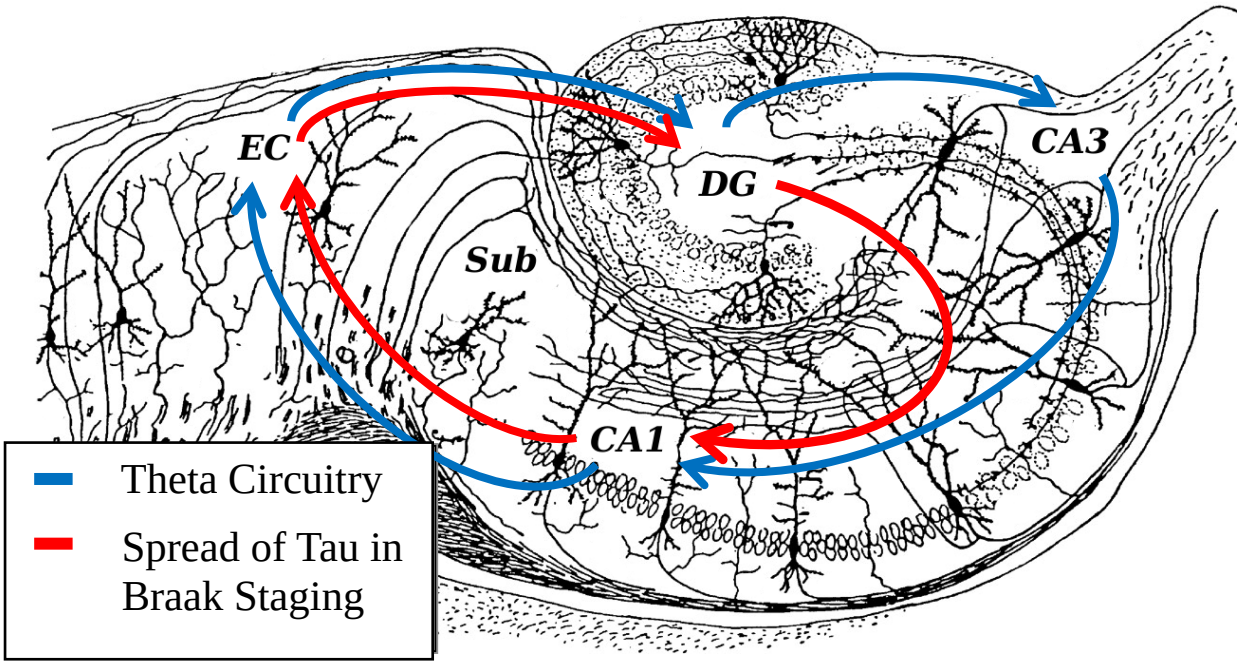

Figure1.5: Theta circuitry (blue arrows) and Tau progression (red arrows) in AD based on Braak staging overlaid on hippocampus drawing by Ramon y Cajal.

As seen in Figure 1.5, the early stages outline by Braak map very closely to the theta circuitry in the hippocampus. Stage I shows NFT changes in the transentorhinal pre-α layer; Stage II is characterized by an accentuation of transentorhinal pathology and the involvement of the main EC region. Together, stages I and II, called the transentorhinal stages correspond to a 'silent' and believed to be pre-symptomatic stage of AD.[70] Notably, this pre-symptomatic stage is affecting the region responsible for driving theta activity—the EC. Subsequently, in Stage III the NFT pathology extends to the dentate gyrus and CA1 subiculum region, further advancing back to the EC (layer V/VI) and begins to emerge in the temporal neocortex.[70] As mentioned above, the dentate gyrus is integral to filtering inputs to the theta circuit and the CA1 region provides feedback to the EC to maintain rhythmicity of the circuit. Stage IV sees NFTs pathology expand to the association areas of the basal neocortex. Stages III/IV, or the limbic stages, correspond to the emergence of impaired cognitive function, so called mild

cognitive impairment (MCI) and mild personality changes. At this stage a person is still not diagnosed with AD. In stage V and VI, the changes extend to the primary areas of the neocortex (stage VI),[70] and it is only at these stages that AD is clinically diagnosed. It is important to note that as the pathology expands, it is also progressively worsening in previously affected areas.

Progression from Stage I to Stage VI can take as long as 10 to 20 years. Therefore, since theta circuitry is impacted early (stages I, II, and III) in pre-symptomatic and mild cognitive impairment stages, theta activity could be a useful tool for earlier diagnosis of cognitive changes with ageing as well as an early therapeutic target. It is also important to note that Braak staging was compiled from the analysis of thousands of individual ageing brains via post-mortem relating NFT pathology to the clinical records. It was not possible to monitor individuals progressively. It is thus probable that a person with Stage I-III early NFT pathology may not advance to later stages of the disease due to lifestyle or other therapeutic interventions—theta frequency modulation could be one of these early therapeutic targets to prevent subsequent decline.

Braak and Braak also detailed the accumulation of Aβ plaques in three stages (A-C). However, there is significantly more inter-individual variability in amyloid plaque deposition and this plaque deposition does not closely align to the clinical symptomology of AD like NFTs. Nonetheless, Aβ plaque buildup impacts many of the same areas, including the EC and hippocampus.[70]

As mentioned above, Aβ and tau, the major proteins associated with memory decline and the progressive destruction of synaptic circuits in AD have a normal function in the brain. These molecules acquire adverse gains of function that progressively impair memory and contribute to neurodegeneration seen in AD. Interestingly both normal and pathological Aβ and tau have been described to regulate and be regulated by theta oscillations.[42,71–74]

Therefore, manipulating theta oscillations could be a way to prevent the Aβ and tau buildup that lead to AD pathology. This is overviewed in sections below.

1.5 Other memory circuitry to be considered: Papez Circuit and Default Mode Network

Neuroimaging, lesion, and electrophysiological studies in humans and animals have led to the overall recognition that memory, and especially episodic memory, is localized to specific brain circuits. This was highlighted, as mentioned above, by patient H.M. who was unable to form new episodic memories after the removal of his hippocampus.[27,28] The ability to definitively identify the brain circuitry and networks that underlie memory and that become impaired during ageing and due to age-related disease, including dementia disorders, is critical. The precise location of this brain circuitry is especially important if we want to target this circuitry to improve memory and ameliorate memory impairment with ageing and in diseases such as AD. Although the precise circuitry remains to be identified, memory and most specifically episodic memory has been localized to two partially intersecting circuits namely, the circuit of Papez and the default mode network (DMN). The human circuit of Papez encompasses the hippocampus, anterior thalamus, mammillary bodies of the hypothalamus, posterior cingulate and fornix.[75] Research at first focused on the Papez circuit's role in emotion, but subsequent studies indicated a role for the circuit in episodic memory,[76–81] including human neuroimaging studies with a focus on AD.[82] The DMN encompasses regions of the brain active at rest, including the medial prefrontal cortex, inferior parietal lobule, hippocampus, posterior cingulate, retrosplenial cortex, and precuneus.[83] The overall resting state connectivity of the adult brain declines as we age, especially within the default mode network (DMN).[84,85] This network is particularly relevant for AD research due to the prominence of Aβ deposition in these regions as well as the importance of these areas for memory.[85,86] This is supported by several studies which implicate altered neural oscillations in the DMN as a very early event in cognitive

impairment that precedes AD.[87–89] The DMN and Papez circuit have overlapping circuitry with each other, namely the hippocampus, which indicates an overlap with the theta circuitry addressed above as well.

Importantly, findings indicate that a theta rhythmic signal may resonate through the Papez circuit, possibly involved in the control of mnemonic functions of the circuit.[90] Lesions of the mammillothalamic tract in the Papez circuit had widespread indirect effects on hippocampo-cortical oscillatory activity within both theta and gamma bands.[91] Studies show that aberrant functional connectivity in Papez circuit correlates with memory performance in middle-aged *APOE4* carriers who do not yet have any cognitive impairment as well.[13,92–94]

Theta activity also displays a relationship with the DMN. Increases in theta power have been associated with a decrease DMN activity.[95] This inverse relationship was also more pronounced for images that were later remembered compared to images that were later forgotten, indicating the importance of this interaction in memory.[96] However, this inverse relationship may be aberrantly exaggerated in AD, with structural and metabolic deficiencies leading to a compensatory increase in theta activity.[97] In fact, individuals in the early stages of AD had more theta activity in regions associated with the DMN compared to individuals with MCI.[98] The combination a decrease in DMN connectivity coupled with an increase in theta activity could be an important marker of AD that reflects underlying memory and theta circuit degeneration. Additionally, age-related differences in DMN connectivity can be altered by using theta-burst stimulation in some adults, providing evidence of a more complex interplay between theta activity, default mode activity and connectivity, ageing, and memory.[99]

While DMN connectivity is typically investigated using fMRI, other studies have characterized how the electrophysiology of the brain changes as we age. Generally, theta

power decreases from young adulthood to middle-age.[25,100,101] Additionally, adults maintaining higher theta power, tend to perform better on cognitive tasks compared to others in their age group.[25,101,102] However, an increase in theta activity is not always considered beneficial. Finnigan et al. differentiate between two types of theta—one associated with healthy cognitive function, and another related to EEG/alpha slowing that reflects a risk for future cognitive decline.[102] Network over-excitation and increased low frequency activity are evident in individuals diagnosed with AD, but it is difficult to determine when the switch occurs and increased theta activity is no longer beneficial. For example, in contrast to the studies linking increased theta power to enhanced cognitive performance, one study found that an increase in theta activity in subjects with mild cognitive impairment (MCI) resulted in a reduction in regional cerebral blood flow.[103]

1.6 Theta oscillations in AD

Alterations in theta frequency activity may be early events that occur pre-symptomatically in AD. Increases in relative theta power have been studied as an early marker of cognitive decline that may lead to AD.[74] Relative theta power was found to be greater in AD cases than healthy controls and relative theta power correlated with total tau in the brain.[104] Furthermore, a correlation was found between cerebrospinal fluid (CSF) total tau, phosphorylated tau and Aβ42 with increased relative theta power and cognitive slowing in healthy elderly adults.[105] Several studies have looked into theta-gamma coupling in the aging brain as well. A relationship between altered theta-gamma coupling and working memory deficits has been demonstrated in individuals with MCI and with AD.[106] Although it is difficult to determine whether network abnormalities or molecular changes in the brain occur first, it is clear they happen in parallel for an extended period of time before cognitive symptoms manifest. These studies indicate that electrophysiological measures of theta could be harnessed as non-invasive early biomarkers for AD.[107] However, it is still difficult to

distinguish between cases of 'healthy ageing' and pathological ageing, making research on middle-aged cohorts especially important.

1.6.1 Theta oscillations and AD: focus on Amyloid-Beta

Overview of Aβ formation

Amyloid beta (Aβ) is a small peptide of sizes varying from 39 to 43 amino acids.[42,108] Aβ occurs normally as a soluble monomer, most commonly 40 amino acids long.[3] However, it can also exist as soluble oligomers or insoluble protofibrils and fibrils[109]—these forms are prominently composed of Aβ 1-42 amino acid form and are most commonly associated with AD.[110,111] To be formed, Aβ must be cleaved from a much larger amyloid precursor protein (APP) by two enzymes, β-secretase and γ-secretase, sequentially.[109,112] APP can also be cleaved by an α-secretase enzyme, but this precludes Aβ formation as it cuts APP right in the middle of the Aβ sequence.[109] Aβ is present in the brains of individuals of all ages, but the amount of Aβ increases as we age, with cognitively intact individuals over 70 having significantly higher levels of Aβ than children and adolescents .[113] The 'amyloid hypothesis' postulated that Aβ peptides came together to form plaques that impaired neurotransmission leading to cell death and cognitive deficits.[110,114] Mutation in the APP or presenilin gene (which encodes the enzyme components of γ-secretase) that cause very rare forms of early onset familial AD (FAD), lead to more Aβ42 buildup and an increased likelihood of developing AD, providing support for the amyloid hypothesis.[59] APOEe4 the major risk factor for sporadic forms of AD can also lead to both increased production and decreased clearance of Aβ.[57,93,115,116]

One major normal physiologic role of Aβ is to depress synaptic activity, protecting against overexcitation.[117] The production of Aβ is also modulated by neuronal excitability.[117] It is difficult to know whether ageing is associated with network over-excitation causing

overexpression of Aβ, or if decreased sensitivity to Aβ leads to its overexpression, or if a completely different factor drives increased Aβ expression as we age. One such possibility is an inability to clear Aβ as it builds up. It may, in fact, be a combination of all of these possibilities. Nevertheless, overproduction of Aβ could lead to adverse suppression of neural activity which is commonly seen in AD.

Aβ and Theta Generation

Aβ, both in soluble and oligomeric forms, is capable of interacting with many molecular targets, making its function difficult to fully understand.[62,118,119] Theta generation is dependent on a balance of excitation and inhibition, and Aβ is capable of increasing excitation as well as decreasing inhibition depending on its molecular target. Conversely it is possible that theta oscillations could drive production of Aβ, block the production of Aβ, or increase/decrease the clearance of Aβ. One study showed Aβ25−35 specifically correlated with a decrease in theta activity in mice, indicating a direct relationship between Aβ and the production of theta oscillations.[120] Additionally, different Aβ peptides can have differing effects on theta activity via specific cellular mechanisms.[120,121] Intra-hippocampal injection of Aβ1-42 reduced theta power in mice completing the Morris water maze and was correlated with poorer memory during the task.[122] A computational model of theta circuitry indicated one of the mechanisms underlying these effects may be the interaction of Aβ with ion channels, including an Aβ-fast-inactivating K^+ channel interaction which induced an increase in theta power.[123] Although the evidence of the direct relationship between Aβ and theta activity regulation needs more investigation, there is considerable evidence of the indirect effects Aβ buildup has on theta generation which is outlined below.

Excitatory neurons that “fire” and release the neurotransmitter glutamate are especially susceptible to degeneration in AD.[57] Aβ and Aβ oligomers are able to interact and

modulate the major glutamate receptors including NMDA (N-methyl-D-Aspartate) and AMPA (α-amino-3-hydoxy-5-methyl-4-isoxazoleproprionic acid) receptor types which are critical for maintaining and mediating the balance of LTP and LTD that underlies learning and memory.[45-47] Multiple studies show normal monomeric forms, but particularly toxic oligomeric and fibrillary forms of Aβ, can impair LTP[124,125]and LTD[126–128] balance . Aβ can also effectively increase the number of synaptic vesicles and amount of glutamate released.[3] Further, Aβ can cause rapid insertion of AMPA receptors that lead to enhanced excitatory postsynaptic currents and disrupt overall calcium homeostasis via NMDA receptors.[129] As explained in sections above, theta oscillations are implicitly linked to the excitatory/inhibitory circuitry underpinning LTP/LTD. Thus, it would be strongly hypothesized that Aβ production and function may be modulated and impacted by theta oscillations both in the "normal'' ageing brain and in AD.

It is important to note that, it is not just Aβ that has physiological activity to regulate excitatory/inhibitory tone in neuronal circuits. Other fragments of APP, particularly soluble forms of APP, sAPPα and APPβ, produced following the α and β secretase "cut" of APP respectively, also regulate the balance between neuronal excitation and inhibition.[130] Recent results reveal that sAPP binds and activates inhibitory GABAB receptors to dampen neuronal "firing".[131] This may be of relevance to the regulation of theta oscillation in the hippocampus where GABAergic interneurons are essential for feedback and synchronisation of theta oscillations in the hippocampus and cortex (see Figure 1.2).

1.6.2 Theta oscillations and AD: focus on tau

Overview of tau and tangle formation

Tau protein occurs naturally in human brains and is classified as a microtubule-associated protein (MAPT) which stabilizes neuronal microtubules particularly in axons.[132] Normally tau is more prominently expressed in the axonal (pre-synaptic) rather than somatodendritic

(post-synaptic) region of neurons. Tau has many additional functions outside its function in microtubule stability as highlighted by Morris et al., in their review "The Many Faces of Tau".[133] It is when tau proteins become misfolded, hyperphosphorylated, and aggregated into NFTs that tau contributes to neurotoxicity by interfering with normal neurotransmission.[132] This occurs in AD and in other neurodegenerative disorders known as tauopathies.[7,133,134] All tauopathies cause dementia, but AD is by far the most common. In AD, tau localizes in NFTs, which are predominantly located in the cell bodies (soma) of glutamatergic pyramidal neurons in the hippocampus and neocortex.[135,136] Additionally, tau can localize in neuropil threads and surrounding plaques (known as neuritic plaques) in AD.[70] Tau also moves from its prominent presynaptic location in the axons to a postsynaptic location in the somatodendritic compartment. This is especially evident within glutamatergic pyramidal neurons in the hippocampus and neocortex in AD.[132,135] Intracellularly, tau has been associated with trafficking of glutamate receptors to the membrane which contributes to altered LTP/LTD balance and plasticity at synapses in AD.[10,137,138] Tau pathology in AD builds up slowly in a stereotypic fashion and correlates with the clinical severity of AD.[52,70] As described by Braak and Braak and described in Figure 1.5 above tau has also been shown to "spread" in a prion like fashion from one neuron to another in neurons overlapping with theta circuitry promoting degeneration and synaptic dysfunction.[139–142] .

The Impact of Tau on Theta Generation

As noted above the developing tau pathological circuitry in the hippocampal formation as described by Braak shows remarkable overlap with hippocampal theta circuitry. This indicates that tau and theta circuitry may have a functional link that is important in the emergence and development of AD. Braak staging details tau buildup beginning in layers II and III of the EC, which contains interneurons that normally synapse with stellate and pyramidal cells in the hippocampus and contribute to theta rhythm generation.[52] Studies have

shown that mice with EC tau build-up displayed higher interneuron firing rates compared to age-matched controls. This increase in firing rate was accompanied by an increase in the theta power as measured by local field potentials (LFPs), indicating that the interneurons from the EC could be driving the increase in theta activity.[143] Together this indicates that tau build-up in AD may increase theta oscillations.

Individuals with the A152T-variant of the tau gene (*MAPT*) display higher levels of tau and increased risk of AD and other tauopathies.[144] Transgenic mice with the same variant (hTau-A152T) exhibited higher theta activity than controls, which can be reversed to normal levels via suppression of hTau-A152T production. However, *MAPT*$^{-/-}$ mice with no tau, exhibited lower levels of theta activity compared to controls, indicating that tau may originally play a role in the production of normal theta oscillations.[144] The prevalence of phosphorylated tau at parvalbumin positive inhibitory interneurons, which are implicated in theta rhythm generation, also signify an effort to combat the increase of theta activity commonly associated with tau buildup.[52] Therefore, tau's contributions to network over-excitation may be due to adverse gain-of-function mechanisms or the promotion and interplay with other potentially detrimental molecules like Aβ.[144]

Generally, tau accumulation in regions of the brain strongly correlates with neuronal and synaptic circuit loss in those areas.[145] On one hand, its presence could be evidence of a neuroimmune response of sorts. On the other hand, it could point toward a more causal role for tau pathology in neurodegeneration. For example, In the absence of Aβ, the injection of seeded tau disrupts network connectivity and induces an inflammatory response that further impairs neural signaling.[145] Even though tau aggregates progressively disrupt brain rhythms and the molecular processes that underlie memory, our understanding of tau build-up and transition from normal soluble form to aggregated forms is still incomplete, which may be contributing to the difficulty of pharmaceutical development. As with Aβ it is possible that

reciprocal alterations in theta oscillations could also drive tau pathogenesis, or that a "feed-forward /feedback" interaction exists between tau and theta oscillations that becomes dysfunctional as the brain progresses from MCI to AD.

1.7 Theta oscillations in relation to inflammation and progression to AD

Microglia are a type of macrophage and are the major immune cells of the brain that, if overactivated, can cause inflammation.[146,147] Astrocytes are also part of the neural immune system, contributing to inflammation as well as essential support to neurons.[148,149] Both microglia and astrocytes work together to maintain neuronal health and efficacy of neurotransmission and neural circuitry. As we age, neurons may become damaged or proteins may be misfolded, activating microglia and astrocytes.[150,151] When functioning normally, microglia are able to clear away misfolded proteins and apoptotic cells while releasing anti-inflammatory cytokines.[152] However, when glial cells, in particular microglia, encounter something like an invading pathogen, they release toxic factors and pro-inflammatory cytokines to kill the pathogen and recruit help.[152] Normally, microglia and astrocytes protect the brain, but when over-activated, they can be dangerous and contribute to neurodegeneration. In AD, over-activation of microglia and astrocytes is closely associated with the developing pathology of the disease.[65–67,153–155]

There are many factors associated with ageing, including telomere shortening, mitochondrial dysfunction, and DNA damage.[156] These changes increase the chances of abnormally folded proteins and neural cell loss. Therefore, it is unsurprising that glial activation and inflammatory markers both increase as we age as well.[152] Furthermore, risk genes for late onset sporadic forms of AD include genes enriched in microglia including TREM2.[157–159] In mouse brains, genes related to inflammation and cellular stress increased with age while genes related to synaptic function decreased.[51] A positive correlation between

DNA methylation in the brain and ageing was also found in humans, indicating how environmental factors may influence brain health later in life.[160]

There are several theories regarding the role of microglia in aging and subsequent disease states. During development, microglia play an important role in the synaptic pruning required for healthy brain maturation.[161] While this pruning typically declines while we age, it could be aberrantly reactivated in AD.[162] Another theory is that, in AD, microglia become dysfunctional as we age and enter into a senescent state, preventing them from effectively clearing plaques.[163] Yet another theory posits that the oxidation of macromolecules prevents their degradation in lysosomes, leading to an overwhelmed phagocytic process as we age.[152]

Regardless of why microglial activation changes as we age, it is evident that both hyperactivation and hypoactivation of microglia can contribute to disease states via promoting apoptosis or failing to clear away protein aggregates.[152,164] Despite the prevalence of both activated microglia and astrocytes in aging and AD, few studies have been done on how neuroinflammation impacts the generation of theta oscillations. In one case, neuroinflammation in a mouse model led to a 50% decrease in hyperpolarization-activated cyclic nucleotide-gated (HCN1)-mediated currents (I_h) in CA1 pyramidal cells, decreasing theta rhythmicity and integrative properties.[165] It is this I_h current that is responsible for tuning the membrane to respond to inputs in the theta frequency range most strongly.[17] Astrocytes were found to modulate theta activity in mice during sleep via Ca^{2+} signalling.[166] Electric stimulation of the septal nucleus was further found to increase Ca^{2+} in hippocampal astrocytes which contributed to LTP of the CA3-CA1 synapses that are integral to theta generation.[167] Furthermore, via the secretion of gliotransmitters, astrocytes have been linked with theta rhythm generation, theta phase precession, and spatial memory in mice.[168] Overall, just as Aβ and tau production may begin as compensatory mechanisms, neuroinflammation and microglial and astrocyte activation may start out as beneficial but can ultimately be

detrimental to brain health. Interactions with theta circuitry may also contribute to this dangerous feedback loop leading to neurodegeneration.

1.8 Neuromodulation Methods

Due to the link of oscillations with a variety of cognitive processes, different methods of altering neural oscillations have been developed for the purpose of treating disease or neuropsychiatric illness.[169–172] Due to the established electrophysiological abnormalities associated with aging and AD, some of these neuromodulation methods could be useful therapeutic options to patients in the future.[173,174]

1.8.1 Deep Brain Stimulation

Deep brain stimulation (DBS) involves implanting electrodes into specific brain areas for repeated electrical stimulation.[175] DBS applied to the subthalamic nucleus in patients with PD can relieve tremors and other movement disorders.[176] When other therapeutic options have failed, DBS can also have beneficial effects for patients with epilepsy and depression.[177,178] While DBS helps some patients, its mechanism is not completely understood. A recent study examined the safety and efficacy of using DBS to relieve symptoms of AD. Stimulation at the ventral capsule/ventral striatum (VC/VS) region in three patients with AD was associated with slower decline in performance on the Clinical Dementia Rating-sum of Boxes measure compared with matched controls with AD.[179] While these results are promising, more research is needed to determine whether the behavioral and cognitive benefits outweigh the risks.

1.8.2 Electrical and Magnetic Stimulation

The less invasive methods of repetitive transcranial magnetic stimulation (rTMS) and transient direct current stimulation (tDCS), can also impact neural activity. In healthy participants, high-frequency rTMS targeted at the hippocampus increased cortical-

hippocampal functional connectivity and improved associative memory.[180] The ability to specifically target the hippocampus with rTMS provided a more direct link between the hippocampus and associative memory. Studies involving Transcranial Electromagnetic Treatment (TEMT) in mouse models of AD seemed to promote 'de-aggregation' of Aβ and tau as well as reverse memory impairment, making this method an exciting treatment option to be explored further in humans.[181] Patients with preclinical AD who received high-frequency rTMS of the precuneus (PC) experienced improved episodic memory and increased beta band activity, indicating magnetic stimulation can favorably impact the electrical activity of the brain.[182] Improved cognitive performance was also found with the use of anodal tDCS over the left inferior frontal cortex in patients with MCI.[183] Though promising, there is a lack of long-term standardized studies to draw conclusions about the efficacy of tDCS. There is a lot of variance among the parameters used for brain stimulation methods, including duration of stimulation, frequency, brain area, length of study, cognitive tests, and participant population.[183]

1.8.3 Sensory Entrainment

Our brain has the natural ability to entrain, or synchronize, to external stimuli like sounds, flashes, or tactile stimuli.[184] To entrain means to 'determine or modify the phase or period of', in this case, brainwaves.[185] Importantly, entrainment serves to modulate activity that is already naturally occurring in the brain via sounds, visual stimuli, or tactile stimulation. It is possible to use this innate ability to alter neural activity in the brain. Almost 20 years ago, J.H. Williams discovered that a 10 Hz flicker improved the memory of healthy individuals and he predicted its future applications in treating memory disorders.[186] Several studies have also examined the impact of rhythmic auditory stimulation on the brain. Infants as early as 7 months of age display frequency-locked EEG rhythms to auditory rhythmic stimuli.[187,188] Many studies have also shown how auditory entrainment is integral to our ability to parse

speech and process sensory stimuli.[189–191] By aligning the phase of neural oscillations to the rhythm of stimuli, entrainment allows for more efficient processing and anticipation of future stimuli.[17]

In addition to sensory processing, auditory entrainment has also been linked with enhanced memory. Hanslmayr et al. recently wrote a thorough review of entrainment and its impact on memory.[192] By modulating sound and luminescence of movies at 4 Hz, either synchronously or asynchronously, 4 Hz neural activity was more strongly entrained in the synchronous condition and the strength of entrainment correlated with associative memory.[193] Not only did the sensory stimuli entrain neural activity, but the behavioral results suggest a role of theta oscillations in binding information from different modalities in memory.[193] This finding makes sense given that sensory information is a key component of episodic memories. In another study, audiovisual stimulation at 5.5 Hz improved the episodic memory retrieval of the context in which words were learned, not just the words themselves.[194] Intracranial EEG recordings during 5 Hz binaural beat stimulation further confirmed the neurological effects of rhythmic auditory stimulation and their association with enhanced long-term memory.[195] Attentive listening nor conscious awareness of auditory stimuli are required for neurological responses either, as demonstrated by sleep studies with auditory stimulation. Auditory stimulation at 12 and 15 Hz successfully increased the number of sleep spindles, or bursts of neurological activity during sleep, which have been linked with memory consolidation.[196] Further, auditory stimulation in phase with ongoing delta waves during slow-wave sleep improved the slow-wave rhythm and consolidation of declarative memory in both young and older participants as well.[197,198] Sensory entrainment is an easy, non-invasive way to modulate neural activity and investigate the function of different frequency bands of activity.

1.9 Justification for Current Study

It is evident even after decades of research, that a comprehensive understanding of what causes and perpetuates AD is still lacking. Overall, it is established that Aβ and tau pathology are present long before cognitive symptoms manifest and are even present in 'healthy' older adult brains. It is also becoming more evident that network dysfunction in the form of abnormal oscillatory activity is also present in very early stages ageing and disease states.[104,199–201] Regardless of whether aberrant network activity or Aβ and tau build-up come first, each feeds back on the other and contributes to cognitive decline.

Recently, Tsai et al. showed that light flickering at 40 Hz entrained gamma oscillations in mice with AD pathology, and repeated stimulation reduced Aβ plaques and increased microglial activity.[202] They further used the same protocol to determine if auditory entrainment with tones at 40 Hz would have a similar effect. They confirmed that auditory entrainment also increased gamma activity and stimulated microglial activity, and visual entrainment used in conjunction with auditory entrainment produced the greatest effect.[203] This finding proves the efficacy of stimulating large neuronal networks via sensory entrainment to induce beneficial molecular changes.

The current study extends this concept of sensory entrainment to improve the memory of humans. Focusing on auditory entrainment to avoid the seizure-inducing effects of flashing light, we aimed to modulate neural activity of middle-aged individuals to promote neural oscillations beneficial to memory. Of the changes associated with ageing, memory lapses are particularly disconcerting and bothersome to many. These changes in memory are also accompanied by changes in neural activity, including a decrease in theta activity.[204] Although memory loss is considered a normal part of ageing, there is a correlation between subjective memory complaints and subsequent development of AD.[205] The current study includes both

individuals with and without memory complaints to determine if there are neurological or behavioral differences between the self-identified groups.

A preliminary study was performed with Dr. Ken Paller and Dr. Jessica Creery, Northwestern University, on younger individuals (mean age 20 years) to affirm the study protocol. Preliminary EEG findings from this earlier study suggested that not every person entrained to the same theta frequency, and the degree of an individual's theta entrainment was correlated with memory improvement.[206] Therefore, the goal of the current study was to extend this study to healthy adults (with and without subjective memory complaints) aged over 50, and to personalize the study to each individual's preferred theta frequency. Sounds at that individual's calculated theta frequency were used for the duration of the study. While their neural activity was recorded using EEG, participants learned and were tested in the completion of a visual spatial memory task, during which each visual object-location was paired with one of three sounds—individualized theta noise, beta-band noise (15 Hz), pure noise. Spatial memory for objects paired with theta noise was compared with memory for objects paired with beta-band noise and pure noise. Induced neural activity was also compared across sound condition.

Hypotheses:

1. Sounds modulated at an individual's preferred theta frequency will increase theta activity in the brain.
2. Objects in the theta condition will be remembered better than those in the other two conditions.

Chapter 2: Methods

2.1 Participants

Ethical approval was obtained for the study (Clinical Research Ethics Committee, University College Cork, ECM 4(k) 03/07/18) Participants were recruited via advertisement both via University College Cork staff email lists, and poster advertisements on campus, in town, and hospital clinics (Appendix i). Exclusion criteria included people aged younger than 50, prior diagnosis of dementia, and previous history of any seizure or 'blackout'.

Forty-seven individuals (30 female, 17 male) aged 50 years or older participated in the study. The participants were aged between 50-78 years (Mean age = 62.3) and screened for significant hearing and eyesight loss with the Etymotic Hearing Test and the Freiburg Visual Acuity and Contrast Test, respectively.[207] Demographic data were collected from each participant, including the participant's education, occupation, and medical history. Cognitive testing was performed using the Montreal Cognitive Assessment tool (MoCA) (Appendix ii).[208] All individuals in the study scored above 24/30 (Mean score = 27.7) and had no prior diagnosis of dementia or mild cognitive impairment. Finally, participants completed the Subjective Memory Complaint Scale (SMC; Appendix iii).[209] Data were collected, stored, and anonymized according to General Data Protection Regulation (GDPR).[210]

2.2 Exclusions

Two participants were excluded from EEG analysis due to recording issues resulting in zero trials for some conditions. Three other participants were excluded due to persistent noise after artefact removal and interpolation of one or more of the eight frontal electrodes (FCz, F3, Cz, F4, FC1, FC2, F1, F2) used to calculate theta power (>2 standard deviations from the mean voltage of the total average of all channels). Final EEG analyses were performed on forty-two participants (25 female, 17 male), with an average age of 62.7 years and average MoCA score of 27.9. Behavioural analyses were completed with all forty-seven participants.

2.3 Procedure

Participants were informed of the study, consented, and provided demographic information. Setting up and preparing participants for EEG recordings took about 45 minutes. A 128-channel active-electrode system (Brain Products ActiChamp128) with EasyCap caps that follow the international 10-20 system were used. An average reference was used during recording. Once EEG impedance fell below the acceptable threshold (< 20 µOhms), the participants began the spatial memory task adapted from Rudoy et al.[211]

2.3.1 Phase 1 - determining preferred theta frequency

During the first phase of the spatial memory task, visual stimuli were presented on a 24" flat panel computer monitor with a refresh rate of 60 Hz. Auditory stimuli were delivered through earphones (Etymonic ER-4, in-ear earphones). The visual stimuli included 120 images of objects (5.3 cm x 5.3 cm) that appeared individually in a random sequence on a computer screen for 2100 ms. A sound began 900 ms before the visual object appeared on the screen resulting in 3000 ms trials (Figure 2.1). The theta frequency is in the 3-7 Hz range. Each object was paired with one of six sounds—pure pink noise, 3-Hz-modulated pink noise, 4-Hz-modulated pink noise, 5-Hz-modulated pink noise, 6-Hz-modulated pink noise, or 7-Hz-modulated pink noise. The sounds were created using Audacity software by modulating the amplitude of pink noise at the selected frequencies. Each sound condition consisted of 20 objects that appeared individually at random locations. Participants were instructed to passively attend to the computer screen while listening to the sounds through earphones.

Immediately after Phase 1, the EEG recording was analysed using EEGlab to determine which frequency of sound induced the strongest increase in theta activity.[212] The EEG data were high-pass filtered at 0.1 Hz and binned according to sound condition. Trials were cut from 1000 ms before the onset of the sound to 3000 ms after the onset of the sound with baseline correction of -750 ms to -500 ms. Trials that exceeded a voltage threshold of

500 mV were excluded. Eight frontal-central electrodes (FCz, F3, Cz, F4, FC1, FC2, F1, F2) were averaged to calculate the frontal midline theta power during each sound condition from 0 to 3000 ms.[213–215] The theta power was calculated at each specific frequency. For example, in the 3 Hz condition, power was calculated from 2.5 – 3.5 Hz then divided by the power between those frequencies in the pure pink noise condition (control condition). The sound frequency with the greatest power increase over the control pink noise condition was selected as the individual's 'preferred theta frequency' and used throughout the rest of the study.

2.3.2 Phase two - Spatial Memory Task

The study phase was the same as phase one except there were only three sound conditions—pure pink noise, 15-Hz-modulated pink noise, and the individual's preferred theta frequency. Each sound condition consisted of 10 objects that had not previously been seen (30 objects total), and participants were told explicitly to try to memorize the object-locations. Again, each trial began with a sound for 900 ms, followed by the presentation of the object on the grid background for the remaining 2100 ms of the 3000 ms trial. The inter-trial interval was jittered with a mean of 1000 ms (800 - 1200). Participants were still only attending to the screen while the object-locations appeared and there were no responses required. The duration of the study phase was about 2 minutes—each of the 30 object-locations was only seen once.

After the 30 object-locations were presented, participants moved into the learning phase. First, the sound played for 900 ms, followed by the appearance of the associated object in the middle of the screen. Participants were instructed to drag the object to its recalled location with the computer mouse. After indicating the recalled location with a left mouse click, the participant received feedback. The object appeared at its correct location accompanied by the associated sound (Figure 2.3). To be considered a correct response, the center of the object had to be less than 150 pixels (5.3 cm) away from the correct location.

After the participant went through all 30 objects, the process was repeated with the same objects in a different random order. When an object was correctly recalled twice, it was removed from the set, and each subsequent run would consist only of the objects that had not garnered two correct responses. After all 30 objects were correctly recalled twice or the 40 minute learning phase time limit was reached, the resting state activity of participants was recorded for 10 minutes (5 minutes eyes open, 5 minutes eyes closed). Participants were randomly assigned to begin with either eyes open or eyes closed resting state. Resting state activity will not be discussed in this dissertation.

Subsequently, participants completed the final test of the 30 object-locations, termed test phase. Like the learning phase, the object appeared in the middle of the screen under the control of the participant's mouse, allowing them to drag the object and left-click to indicate the recalled location. This time, there was no feedback, and the participants only went through the 30 objects once.

2.4 Data Processing

2.4.1 Behavioural Data:

The distance formula between the correct location (x_1, y_1) and the recalled location (x_2, y_2) $d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$ was used to calculate the recall error during the learning and test phases. Average error was calculated for each sound condition within each subject. To account for increased difficulty of object-locations closer to outer edges compared to object-locations closer to the center of the grid, a 'memory score' was calculated similar to Jacobs et al.[216] The recall error for each participant was compared against a distribution of all possible errors for the correct location and given a percentile rank. The memory score was calculated by subtracting the percentile rank from 1. Therefore, a higher memory score indicated higher accuracy. Additionally, the average number of learning trials per sound condition was calculated for each participant.

2.4.2 EEG Data and EEG data analysis

The data were recorded with a bandpass of 0.1-100 Hz at a sampling rate of 1000 Hz. Electrodes were placed at 128 standard scalp locations. The data were then processed offline using EEGlab and Fieldtrip software.[212,217] First, large muscle and jump artifacts were rejected using a z-score threshold using the automatic artefact reject procedure in Fieldtrip.[217] Then, independent component analysis (ICA) was used to remove blink and rolling eye movement artifacts.[218] Noisy channels were identified via visual inspection of the butterfly spectra and interpolated using a weighted average of nearest neighbouring electrodes. The data were re-referenced to the average reference and detrended. Trials for each participant were binned according to Phase (study, learning, test), which were further subdivided into sound condition (pure, beta, theta). The data were epoched from 1000 ms before to 3000 ms after the onset of the sound—the visual object appeared at 900 ms. Trials and channels were visually inspected for a final time to reject any outlying trials and identify channels that were still noisy after channel interpolation. Five channels (F6, O1, POz, P3, Pz) were not included in time frequency analyses due to excessive noise after interpolation. Time-frequency spectra (1 – 30 Hz) were calculated for each trial -500 ms to 2000 ms to avoid movement activity in the learning phase. A baseline correction of -750 ms to -500 ms was used so anticipatory effects could still be examined. Subsequently, grand-averages were calculated for three separate memory groups according to SMC scale scores (0-3 = none, 4-6 = mild, 7-12 =moderate/severe).

Time-frequency representations (TRFs) were computed by means of Hanning windows with a frequency–dependent width, at frequencies between 2 to 30 Hz, in 2 Hz steps. Additionally, broad-band signals from 30-120 Hz were multi-tapered in steps of 2 Hz with a width of 5 cycles per time window. Data was normalized using an absolute baseline correction before computing grand averages over all participants.

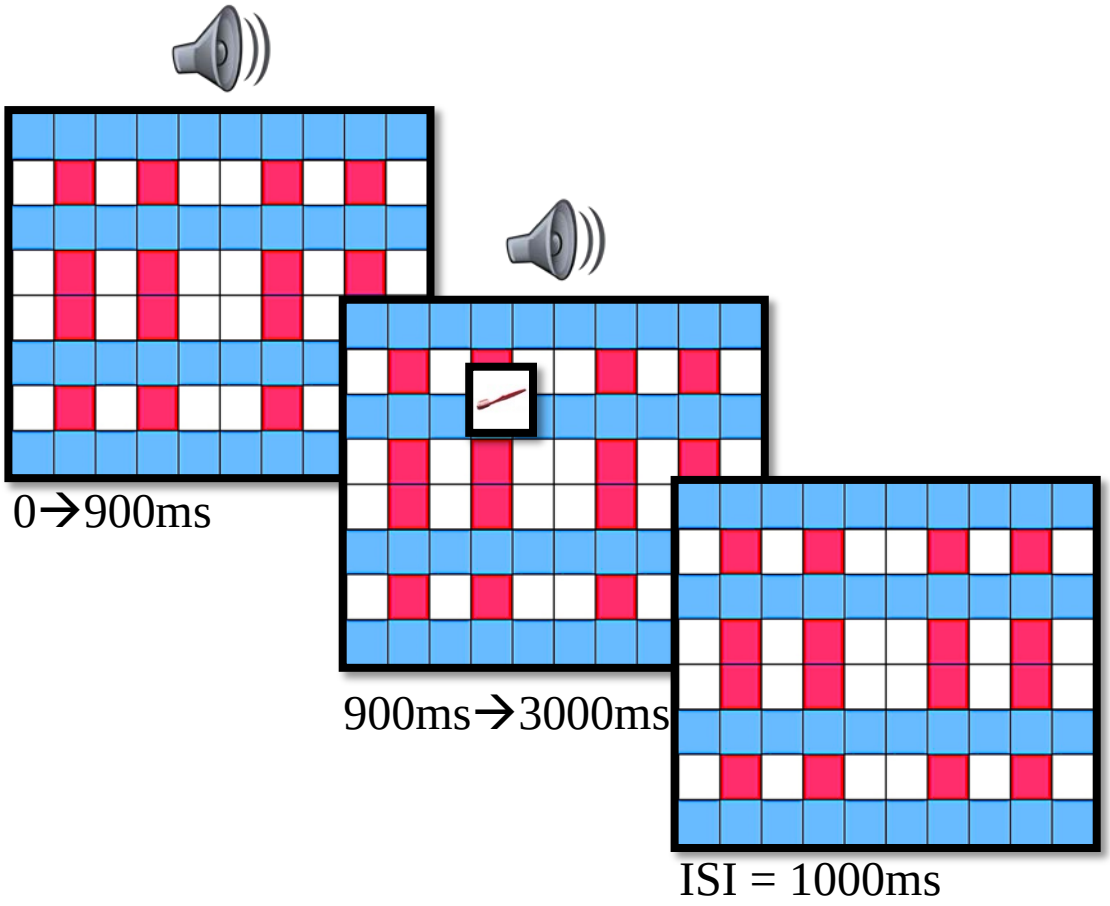

Figure 2.1: Phase 1. One of three sounds was played for 900 ms before the object appeared at a random location on a grid background. The Image and sound were presented together for the remainder of the 3000ms trial. After jittered inter-stimulus interval (ISI) with a mean of 1000ms, the next trial began, this continued until 120 object-locations had been seen.

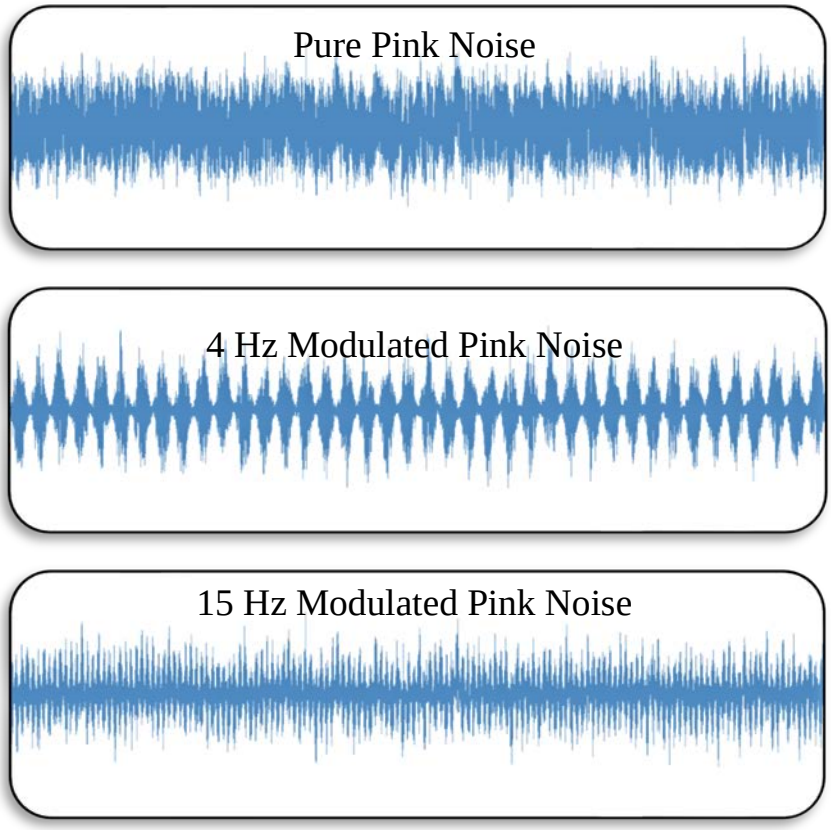

Figure 2.2: An example of sounds an object-location could be paired with if a participant's preferred theta frequency was 4 Hz. Ten object-locations were randomly paired with each type of sound at the beginning of the phase two and remained paired for the duration of the task.

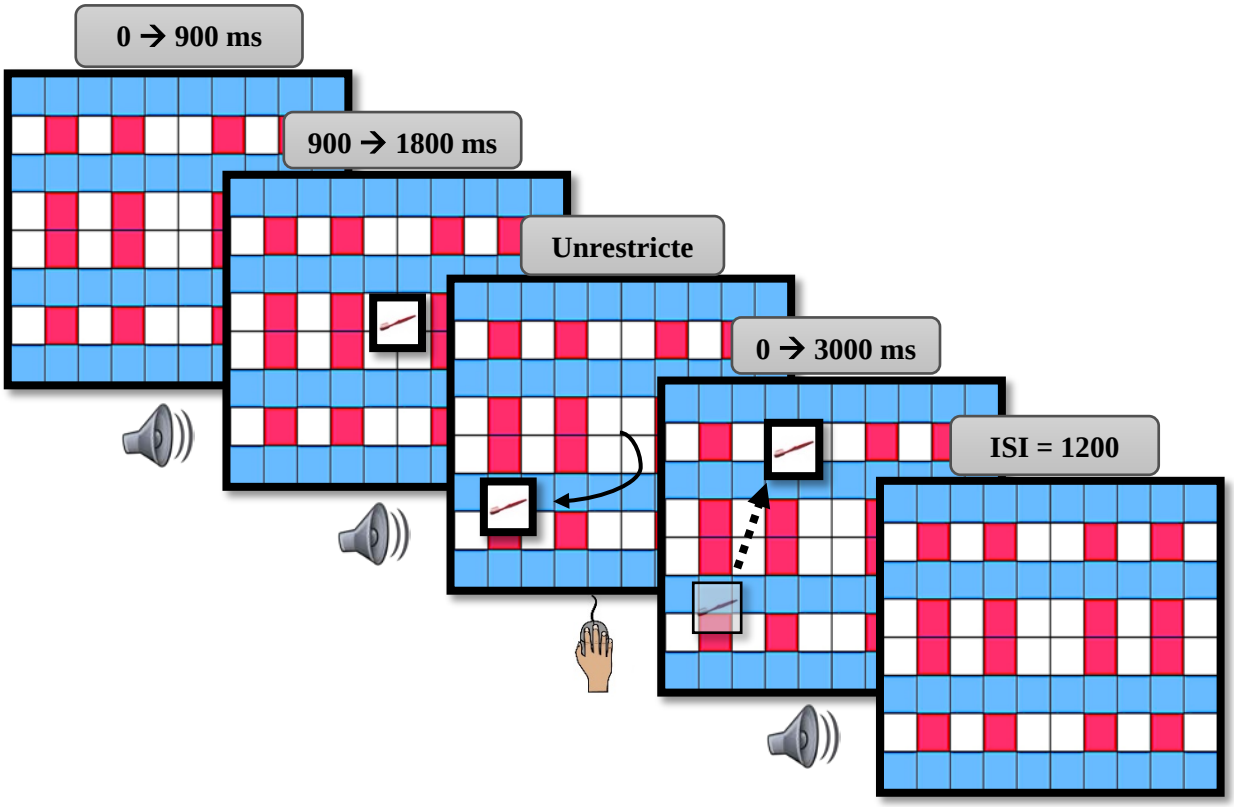

Figure 2.3: The learning phase begins with 900ms of one of the sounds followed by the appearance of an object in the middle of the grid screen. The object remains locked in the middle of the screen accompanied by the sound for 900ms. At 1800 ms the object is under the control of the participant's mouse and they have unlimited time to drag the object to where they think it belongs. After the participant clicked the mouse to indicate the recalled location, they received feedback and the image appeared in its correct location accompanied by the associated sound for 3000 ms. A jittered inter-stimulus interval of 1200 ms occurred before the next trial began.

Chapter 3: Results

3.1 Behavioural Analysis

Based on their score from the Subjective Memory Complaint Scale (Appendix iii), participants were classified as having no memory complaints (score 0-3), mild memory complaints (score 3-6), or moderate/severe memory complaints (score > 6). Moving forward, these groups will be referred to as No MC, Mild MC, and Moderate MC. The groups had similar average ages: No MC (n =15) average age of 61.9 years, Mild MC (n =16) average age of 62.6 years, and Moderate MC (n =16) average age of 62.3 years. All individuals had a MoCA score of 24 and above with group average scores of: No MC = 28.6, Mild MC = 28.0, Moderate MC = 26.9. There was a significant difference in average MoCA score between groups (One-way ANOVA $F(2,44) = 4.727$, $p = 0.014$) (Figure 3.1). A Tukey post hoc test showed the Moderate MC group had a significantly lower average MoCA score compared to the No MC group ($p = 0.012$), while the other group differences were not significant (No MC x Mild MC: $p = 0.550$; Mild MC x Moderate MC: $p = 0.124$).

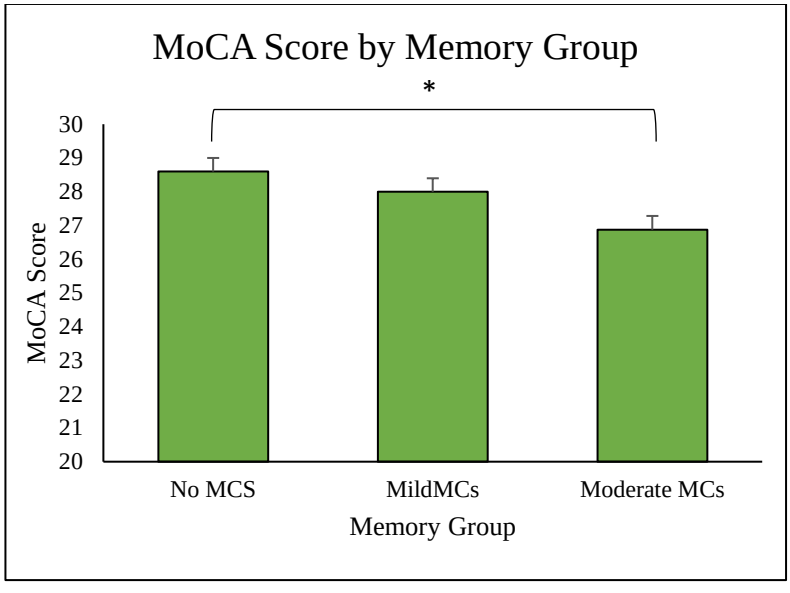

Figure 3.1: MoCA Score by Memory Group. Individuals in the No MC group scored significantly better on the MoCA compared to individuals in the Moderate MC group. The (*) indicates significant difference between the No MC and Moderate MC group MoCA score (p <0.05).

Prior to the memory task, each participant's preferred theta frequency was calculated. As predicted, not all participants preferred the same theta frequency: 3 Hz (n = 9), 4 Hz (n = 6), 5 Hz (n = 9), 6 Hz (n = 13), 7 Hz (n = 10). Welch's two-tailed t-tests were used to compare the average preferred theta in the three memory groups. There was a significant difference in the preferred theta frequency between the Mild MC group (M = 5.8, SD = 1.2) and the Moderate MC group (M = 4.8, SD = 1.5); t(29) = 2.05, p = 0.05, g = 0.72, where the preferred theta frequency was higher in the mild compared to moderate group. The average preferred theta of the Mild MC group was also close to being statistically different than the No MC group (M = 4.9, SD = 1.4) as well; t(28) = 1.9, p = 0.07, g = 0.67. There was no significant difference in preferred theta between the No MC group and the Moderate MC group; t(29) = 0.2, p = 0.8, g = 0.08 (Figure 3.2).

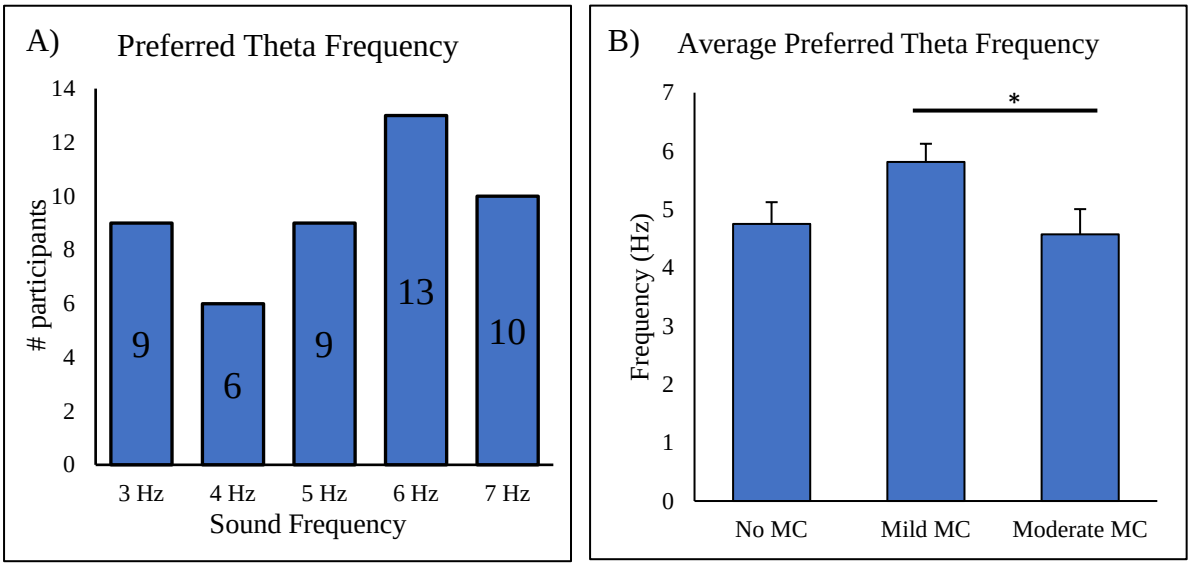

Figure 3.2: Differences in Preferred Theta Frequency. A) The left graph displays the number of participants that preferred each specific theta frequency. B) The right graph depicts the average preferred theta frequency for each MC group, (*) indicates significant difference in preferred theta frequency between the Mild and Moderate MC groups (p = 0.05) .

After preferred theta frequency was calculated, participants began the memory task portion of the experiment. After being exposed to all of the object locations in the study phase, participants moved into the learning phase where they were able to interact with the

objects. There was no significant difference in average error for the first learning trial between or within memory groups (Figure 3.3).

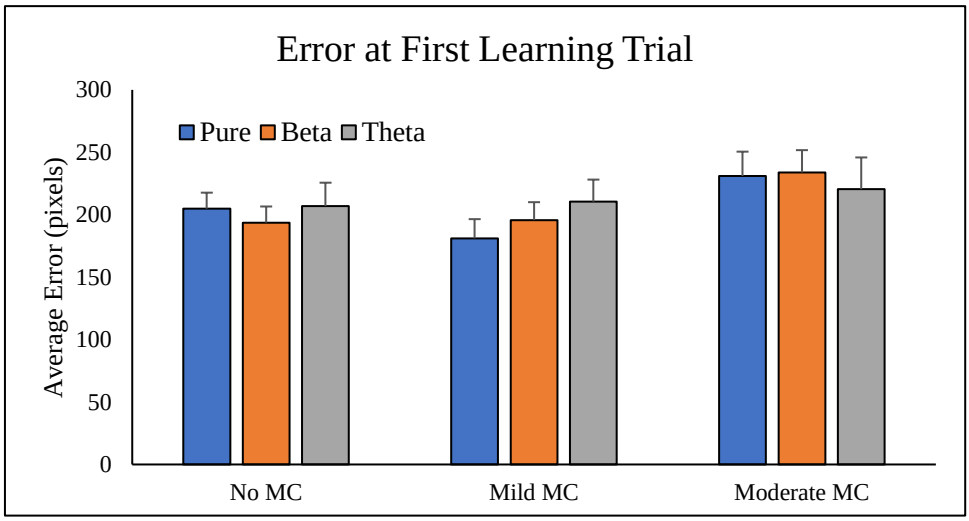

Figure 3.3: Error at first learning trial. There were no significant differences in error at the first learning trial between or within the three MC Groups.

The number of learning trials required to learn object-locations up to criterion was also measured using a 3 x 3 mixed design ANOVA with MC Group as the between-subjects factor (No MC, Mild MC, and Moderate MC) and Sound Condition (pure, beta, theta) as the within-subjects factor. There was no main effect between groups (No MC x Mild MC, $p = 0.68$; No MC x Moderate MC, $p = 0.40$; Mild MC x Moderate MC, $p = 0.81$). However, there was a significant interaction between Group and Sound Condition with a difference between the number of learning trials required for beta ($M = 57.1$. $SD = 20.4$) and theta ($M = 49.2$, $SD = 20.2$) conditions which was most prominent in the Moderate MC group (Student's two-tailed t-test, $t(15) = 3.0$, $p = 0.01$, $d = 0.39$) (Figure 3.4). Together, these results suggest that the Moderate MC group was able to learn the object-locations in the theta sound condition faster than in the beta condition.

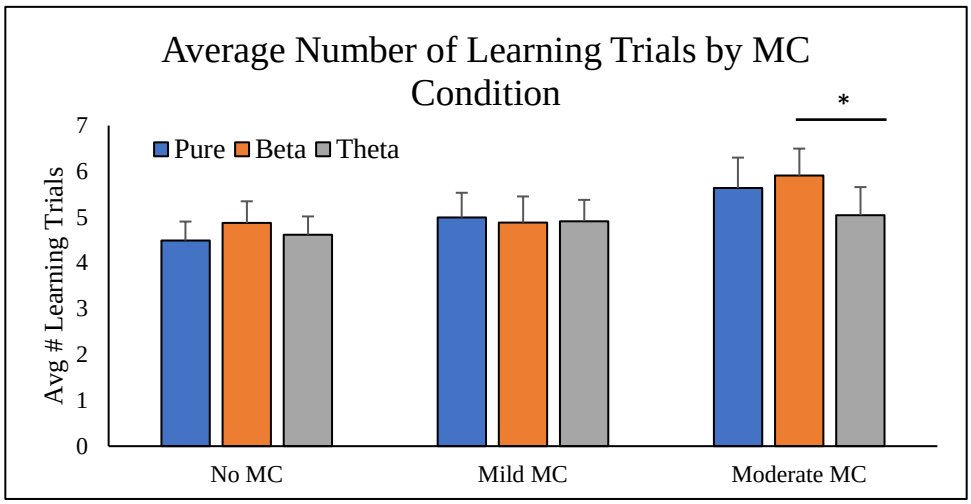

Figure 3.4: Average number of learning trials required to learn object-locations to criterion. The Moderate MC Group required significantly more learning trials (P < 0.05) in the beta condition compared to the theta condition. This difference was not present in the other MC Groups.*

After the final test, average memory scores were compared between groups with a 3 x 3 mixed design ANOVA (between-subjects factor: MC Group, within-subjects factor: Sound Condition). Individuals in the No MC and Mild MC groups performed on average 5.4% and 5.5% better respectively than the individuals in the Moderate MC group (No MC x Moderate MC, $p = 0.046$; Mild MCs x Moderate MCs $p = 0.026$; No MC x Mild MC, $p = 0.972$). A factor MANOVA displayed that this group difference was most evident in the theta condition (MC Group x Theta $(F44,2) = 4.15$, $p = 0.022$), with the largest difference between the Mild and Moderate MC groups (Tukey HSD mean difference = 0.085, $p = 0.017$). The difference in memory score for the beta condition between the No MC group and Moderate MC Group was also close to being significant (Tukey HSD mean difference = 0.0812, $p = 0.06$). These data indicate the Moderate MC group displayed overall worse memory for object-locations (Figure 3.5a), and their memory for object-locations paired with theta sounds was especially poor compared with the Mild MC group (Figure 3.5b).

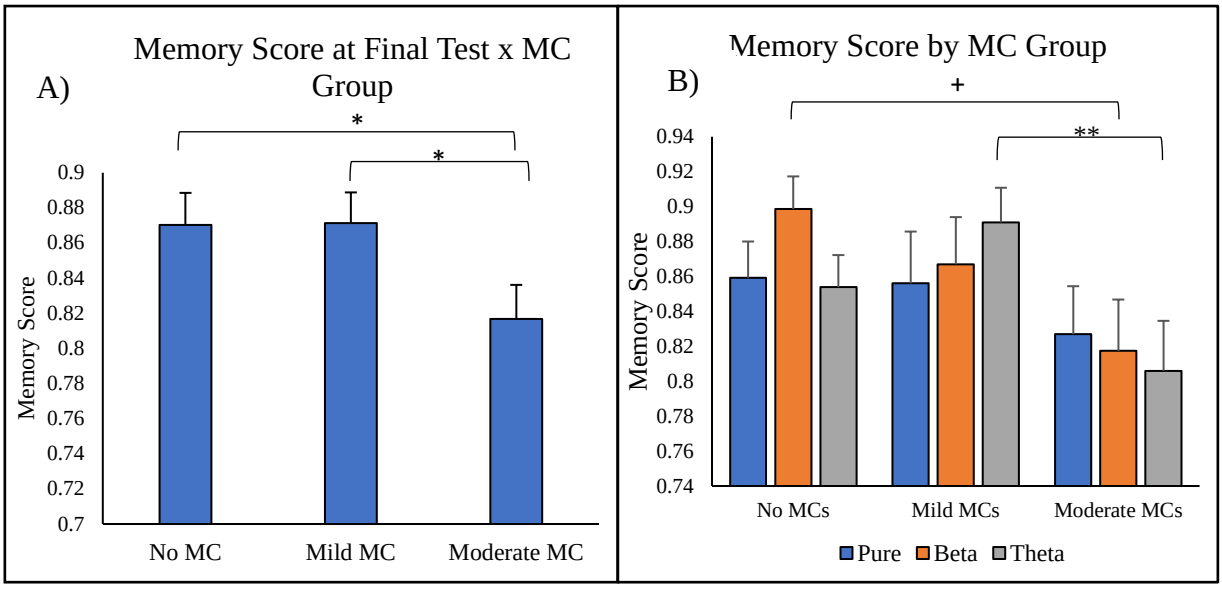

Figure 3.5: Memory Score Differences by MC Group. A) ***Left*** *Graph shows average memory scores for each MC group, with the No MC and Mild MC groups displaying higher memory scores than the No MC group (*P < 0.05). B)* ***Right*** *graph shows the average memory group separated out by MC group and sound condition. The Mild MC group displayed higher memory scores in the theta condition compared to the Moderate MC group (**P < 0.01). The No MC group displayed close to significantly better memory for objects in the beta sound condition compared to the Moderate MC group ($^{+}P = 0.06$).*

Additionally, when sorted by preferred theta frequency, the average memory score was significantly lower in individuals that preferred 4 Hz compared to 6 Hz only within the theta condition (One-way ANOVA, F = 3.268, p = 0.022; Multiple comparisons Tukey HSD, p = 0.026) (Figure 3.6). This could indicate higher theta frequencies are better for memory. However, the small group size in the 4 Hz group compared to the 6 Hz group could be contributing to this difference (n = 6 vs n = 13).

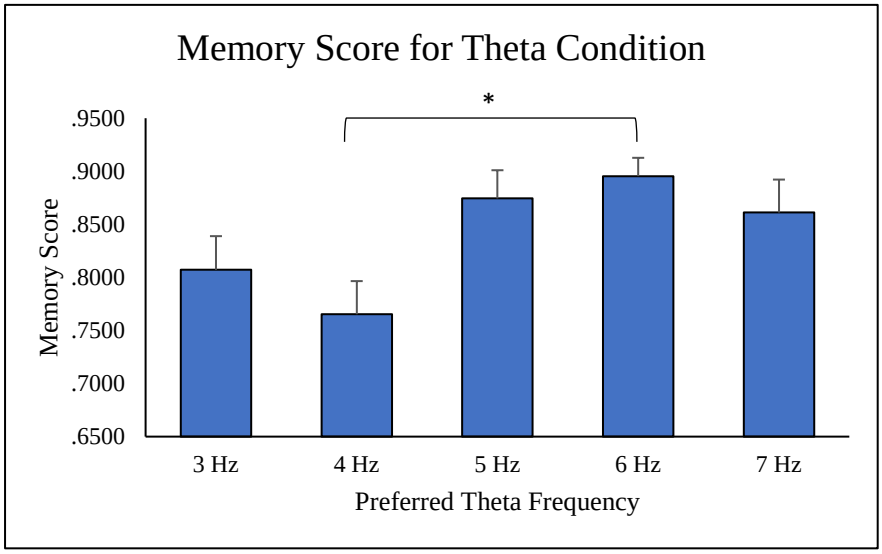

*Figure 3.6: Differences in memory score based on preferred theta frequency. Individuals that preferred 6 Hz sounds displayed significantly higher memory scores compared to individuals that preferred 4 Hz sounds (*P < 0.05).*

There was also a significant difference in memory score between males and females. Males had a significantly higher memory score, but only within the theta sound condition (MANOVA $F(44,2) = 4.878$, $p = 0.032$) (Figure 3.7). There was no difference in average age between males (60.3 years) and females (63.4 years) and there was a similar distribution among MC groups as well, indicating some other factor must be causing the memory difference.

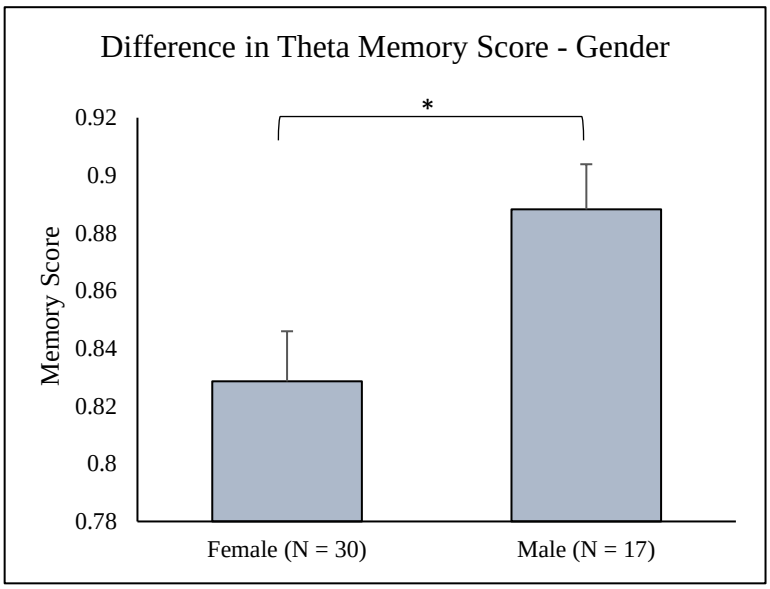

*Figure 3.7: Differences in memory score in the theta condition based on gender. Men displayed significantly higher memory scores than women (*P < 0.05).*

3.2 EEG Analysis

During the study phase, when participants were passively attending to the object-locations while listening to sounds, there was a trend toward more theta activity while the participant's preferred theta sound was playing compared to when beta or pure sounds were playing (MANOVA: theta sound condition x pure sound condition: mean difference = 0.158, p = 0.09; theta sound condition x beta sound condition: mean difference = 0.158, p = 0.07; pure sound condition x beta sound condition: mean difference = 0, p = 0.998). The activity in 8 frontal-central electrodes (F3, FC1, Cz, FC2, F4, F1, F2, FCz) was averaged for this analysis

due to noise in other channels and past precedence in analysing frontal-midline theta (Figure 3.8).[36,100,219]

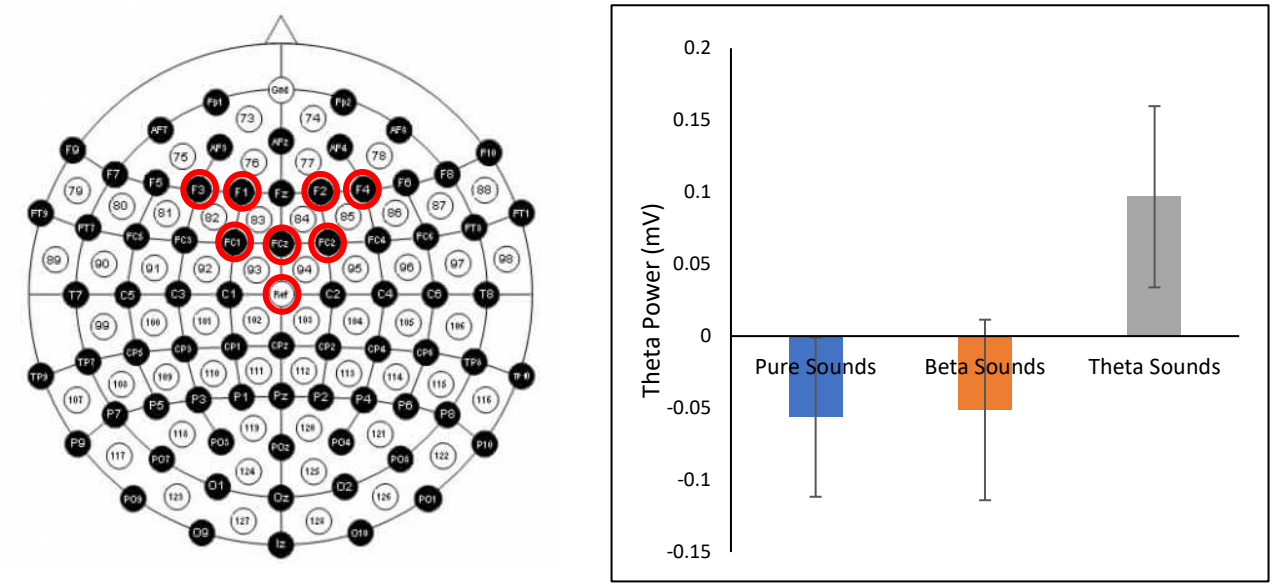

Figure 3.8: ***Left****) A topographical representation (triangle at top centre indicates front of head) of the 8 frontal-central electrodes used for the power analysis shown on the right. Red circles indicate the location of the eight electrodes used for further analysis.* ***Right****) Overall theta power (3-7 Hz) during the Study Phase for the three sound conditions. There was a trend toward increased theta power while theta sounds were playing compared to the other two sound conditions which displayed decreased theta power.*

3.2.1 EEG Differences Between Memory Groups

For all EEG analyses, only induced activity was analysed. A 3 x 3 x 2 mixed-design ANOVA within group (No, Mild, Moderate) as the between-subject factor and Sound Condition (Pure, Beta, Theta) x Experimental Phase (Study and Learn) as the within subjects factors, was performed on the pre-processed EEG data across all electrodes. The test phase was not included in EEG analysis due to variable time scales across individuals—participants were given as much time as necessary to recall the location of each object. Data were analysed from 500 ms before the onset of the auditory stimulus until 2000 ms post-stimulus. Theta power (3 – 7 Hz) was averaged for each time point within each condition for every participant. To correct for multiple comparisons (i.e., 128 channels by 250 time points) a cluster-based permutation test (Monte Carlo simulations) was used.[220] Clusters were created based on F-values that exceeded a critical threshold of $p \leq 0.05$ for adjacent grid points. The

cluster statistic was calculated from the sum of the F-values within a specific cluster. These cluster values were then compared to the cluster values calculated from 1000 permuted data sets, only those with an alpha value < 0.05 were designated as significant.[221,222] Additional post-hoc comparisons were calculated by two-tailed dependent t-tests (2000 permutations, cluster-alpha = 0.05). A significant cluster of occipital-parietal electrodes showed a main effect of memory group on theta activity from 1.1 s to 1.6 s.

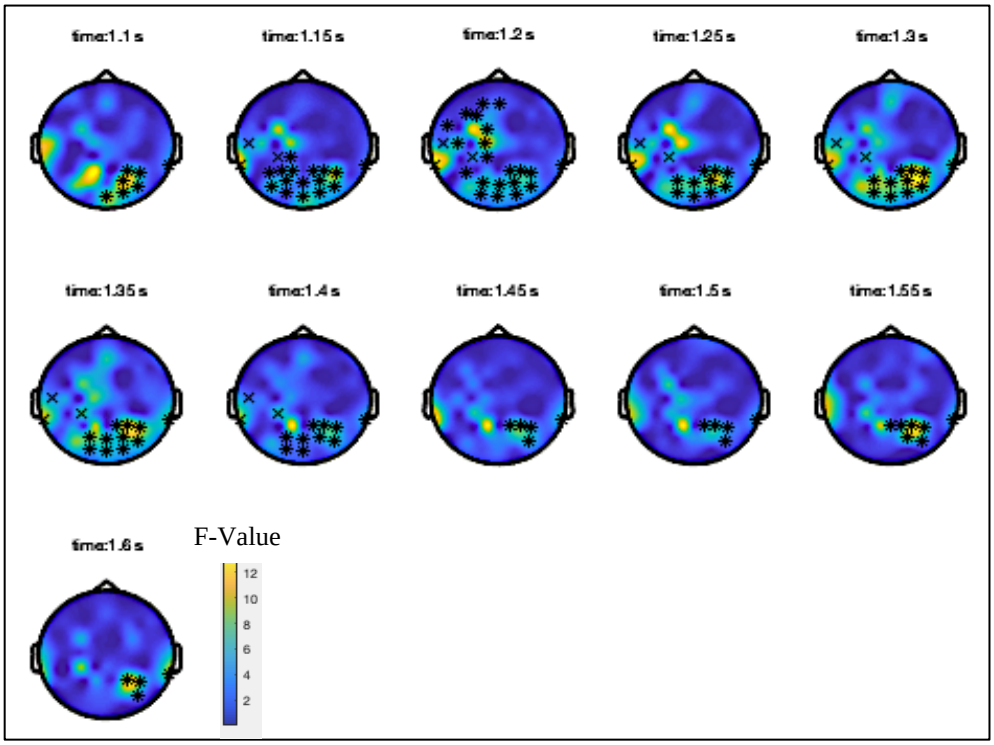

Figure 3.9: Topographical distribution of significant cluster associated with the main effect of MC group on 3-7 Hz activity. Theta band power differed between groups in occipital-parietal sensors. The color bar represents F-values of the ANOVA for each topographical plot (yellow represents a higher F-value and blue represents a lower F-value). Significant channels ($p < 0.05$) are marked with (*), sensors close to significance $p < 0.09$ marked with (x).

The topological plots in Figure 3.9 display the channels that showed a significant main effect of group when comparing theta power distributions. These clusters do not necessarily mean that changes in theta activity are localized to those spatial locations but are rather an indication of general difference between groups.[220,223] To further elucidate this group effect neural activity, average time-frequency spectra were analysed between MC groups as outlined in sections below.

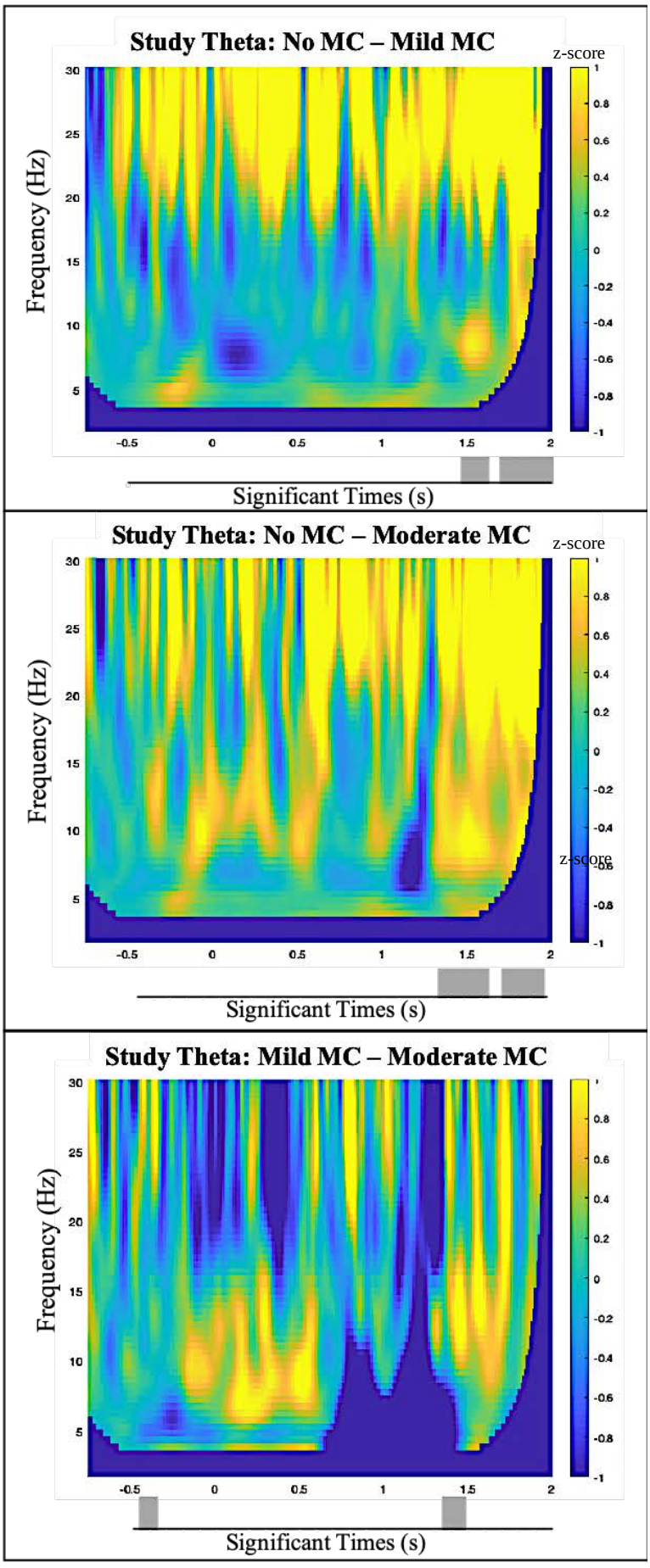

Figure 3.10: Time frequency spectral power differences between MC groups averaged over the theta sound condition of the study phase. Times associated with significant differences in theta (3- 7 Hz) power are marked with grey boxes below the x-axis (P <0.05). ***Top****) No MC – Mild MC difference plot, color bar represents z-scores of power values (yellow indicates higher power in the No MC group, dark blue indicates higher power in the Mild MC group).* ***Middle****) No MC – Moderate MC difference plot (yellow indicates higher power in the No MC group, dark blue indicates higher power in the Moderate MC group.* ***Bottom****) Mild MC – Moderate MC difference plot (yellow indicates higher power in the Mild Mc group, dark blue indicates higher power in the Moderate MC group). The largest differences in theta power between groups were seen post-visual stimulus presentation at t > 1 s.*

T-test Groups: Study Phase	Times with significant differences in theta power (s)	Significant frequencies averaged over time (Hz)
No MC x Mild MC	1.48 – 1.62; 1.71 – 2.0	4.0; 20,0 – 30.0
No MC x Moderate MC	1.35 – 1.62; 1.74 – 1.96	4.0 – 14. 0; 21.0 – 30.0
Mild MC x Moderate MC	-0.45 to -0.36; 1.36 – 1.47	12.0 – 13.0

Table 3.1: T-tests between MC group in the theta condition of the study phase. The first column reiterates the time points when theta activity significantly differed between MC group indicated with grey bars in figure 3.10 (P < 0.05). The second column is the result of averaging over time to examine which frequencies besides the theta band differed between groups (P < 0.05). The No MC group exhibited significantly more 4 Hz theta power as well as high (20 – 30 Hz) beta power compared to the other groups. While the Mild and Moderate MC groups only differed at alpha frequencies (12 – 13 Hz).

Again, these spectra are the result of averaging over the same 8 frontal-central electrodes in Figure 3.8 (F3, FC1, Cz, FC2, F4, F1, F2, FCz). At time = 0, the auditory stimulus began (in this case, the individual's preferred theta frequency), and time = 1 represents when the image of the object appeared on the screen. Activation within the theta band at time 0 and time 1 can be seen in all MC groups to varying degrees. Independent Samples t-tests were performed with Monte Carlo correction to determine time points when theta activity (averaged over 3 - 7 Hz) was significantly different between groups. The greatest difference was between the No MC group and Moderate MC group which significantly differed in theta activity from 1.35 – 1.62 s ($p < 0.05$). The No MC group differed from the Mild MC group in theta power from 1.48 – 1.62 s ($p < 0.05$). The Mild MC Group and Moderate MC group differed for the fewest time points, 1.36 – 1.47 s ($p < 0.05$).

Upon seeing the high beta power in the No MC time frequency spectra, subsequent independent samples t-tests were performed averaged across time to determine which individual frequencies (1 – 30 Hz) differed significantly between MC Group. The No MC group differed from the Mild MC group at 4 Hz and 20 – 30 Hz ($p < 0.05$). These differences are evident in Figure 3.10, with the No MC power spectrum displaying higher beta activity and lower theta activity than the Mild MC group. Similarly, the No MC group also had significantly more power in the high beta range (21 – 30 Hz) compared to the Moderate MC group, in addition to higher alpha/low beta activity (8 – 14 Hz) ($p < 0.05$). The only

significant difference between the Mild MC group and Moderate MC group was from 12 – 13 Hz ($p < 0.05$). The reasons why such robust changes in alpha and beta activity can be seen while theta sounds were playing is considered in the discussion section.

To determine if these patterns persisted, the same independent-samples t-tests with Monte Carlo cluster correction were performed on the EEG data (8 frontal electrodes from Figure 3.8) from the three MC Groups during the learning phase. Data were again analysed from 500 ms before the onset of the auditory stimulus to 2000 ms after, which corresponds to when the participant was able to drag the object to the recalled location. Time = 0 s in Figure 3.11 reflects the onset of the auditory stimulus and time = 1 s is when the image of the object appears in the middle of the screen. Time 1 – 2 s is when participants are trying to recall the object-location, but the image is still locked in the middle of the screen, so this time should be unaffected by motor artifacts.

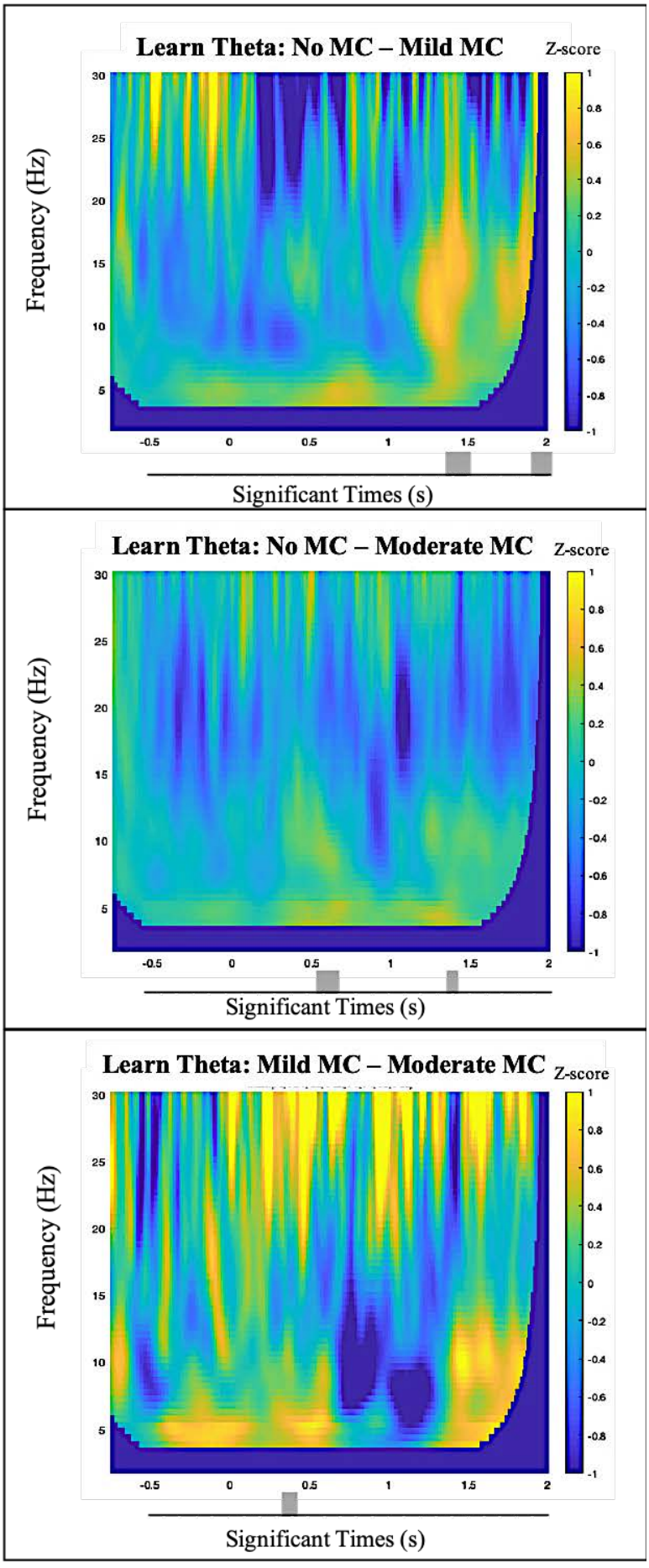

Figure 3.11: Time frequency spectral power differences between MC groups averaged over the theta sound condition of the learning phase. Times associated with significant differences in theta (3- 7 Hz) power are marked with grey boxes below the x-axis (P <0.05). ***Top****) No MC – Mild MC difference plot, color bar represents z-scores of power values (yellow indicates higher power in the No MC group, dark blue indicates higher power in the Mild MC group).* ***Middle****) No MC – Moderate MC difference plot (yellow indicates higher power in the No MC group, dark blue indicates higher power in the Moderate MC group).* ***Bottom****) Mild MC – Moderate MC difference plot (yellow indicates higher power in the Mild MC group, dark blue indicates higher power in the Moderate MC group). This plot looks like it shows the largest difference between groups, but high levels of variance could be contributing to lack of more significant time points.*

T-test Groups: Learning Phase	Times with significant differences in theta power (s)	Significant frequencies averaged over time (Hz)
No MC x Mild MC	1.37 – 1.46; 1.91 – 2.0	none
No MC x Moderate MC	0.56 – 0.69; 1.36 – 1.41	13.0 – 20.0
Mild MC x Moderate MC	0.35 – 0.41	13.0 – 19.0

Table 3.2: T-tests between MC group in the theta condition of the learning phase. The first column reiterates the time points when theta activity significantly differed between MC group indicated with grey bars in figure 3.11 ($P < 0.05$). The second column is the result of averaging over time to examine which frequencies besides the theta band differed between groups ($P < 0.05$). The No MC group exhibited significantly less low beta activity (13 – 20 Hz) compared to the Moderate MC group. The Mild MC group also displayed significantly lower low beta activity (13 – 19 Hz) compared to the Moderate MC group ($P < 0.05$). These results indicate an increase in low beta activity in the Moderate MC group during the theta condition of the learning phase.

During the learning phase (Figure 3.11, Table 3.2), the No MC group displayed significantly higher theta activity than both the Mild and Moderate MC groups from about 1.37 s to around 1.41 s ($p < 0.05$). There was also a difference between the Mild and Moderate MC groups before the visual stimulus was presented from 0.35 to 0.41 s ($p < 0.05$). We expected to see a larger difference between the Mild and Moderate MC groups, but there was low statistical power due to increased variance.

Once again, we wanted to look at other frequency changes with independent samples t-tests averaged across time to determine which individual frequencies (1 – 30 Hz) differed significantly between MC Group. In contrast to the study phase, the Moderate MC group exhibited significantly more low beta activity (13 -19 Hz) than both the Mild and No MC groups ($p < 0.05$).

Thus far, the main effect of Group on theta activity drove us to more closely examine the time-frequency spectra of the three MC Groups during the theta condition of the study phase (Figure 3.10) and learning phase (Figure 3.11). While average theta activity differed across several time points between MC Groups, the spectra also revealed differences in alpha and beta activity between MC Groups in the theta condition.

Another 3 x 3 x 2 mixed-design ANOVA with MC Group (No, Mild, Moderate) as the between-subject factor and Sound Condition (Pure, Beta, Theta) x Experimental Phase (Study and Learn) as the within subjects factors, was performed averaged over the alpha band (8 – 12 Hz). The cluster-based permutation tests revealed a main effect of memory group on alpha power ($p < 0.05$; see Figure 3.12).

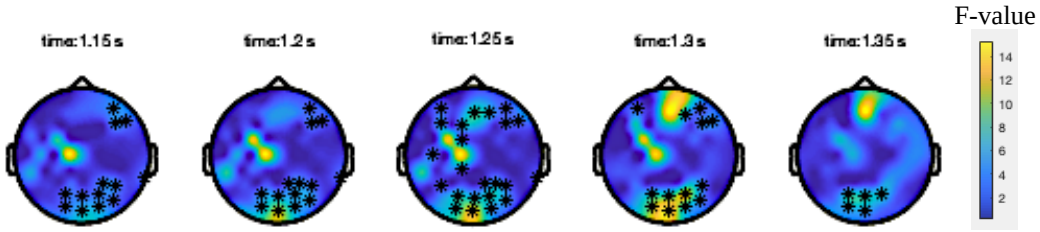

Figure 3.12: Topographical distribution of significant clusters associated with the main effect of MC group on 8-12 Hz activity. Alpha band power differed between groups in occipital and frontal sensors. The color bar represents F-values of the ANOVA for each topographical plot (yellow represents a higher F-value and blue represents a lower F-value). Significant channels ($p < 0.05$) are marked with (*),

Additionally, when averaging over the beta band (12 – 30 Hz), the cluster-based permutation tests revealed a near significant main effect of Group and beta power ($p = 0.08$).

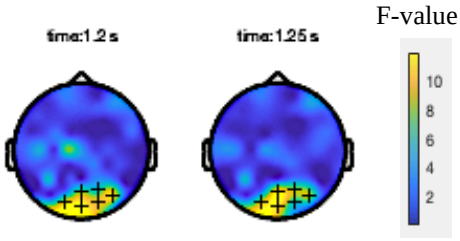

Figure 3.13: Topographical distribution of significant cluster associated with the main effect of MC group on 13-30 Hz activity. Beta band power differed between groups in occipital sensors. The color bar represents F-values of the ANOVA for each topographical plot (yellow represents a higher F-value and blue represents a lower F-value). Channels close to significance ($p < 0.08$) marked with (+).

These findings suggest that the neural activity of the three MC groups significantly differed from one another. Additionally these differences in EEG activity were not restricted

to the theta band, but were also present in the alpha band and even the beta band to some extent.

3.2.2 EEG Differences Within MC Groups Based on Sound Condition

Cluster-based permutation dependent samples t-tests were done within each MC group to determine if there was more theta activity during the theta sound condition compared to the beta and pure sound conditions. The Moderate MC Group was the only group that exhibited clusters showing a significant increase in theta power between the theta condition and beta condition during the study phase (Figure 3.14).

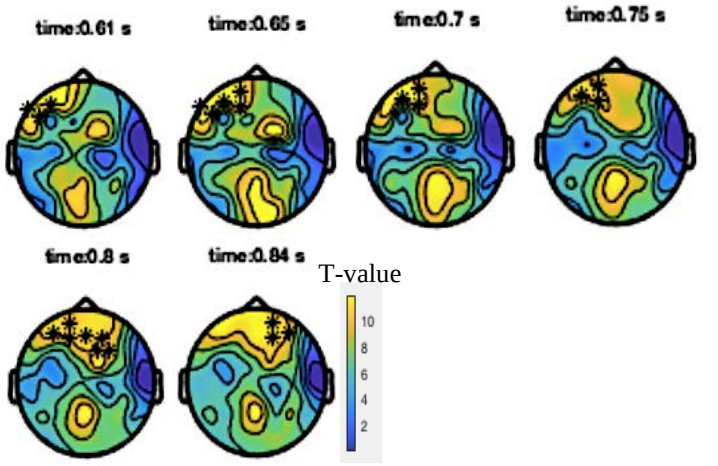

Figure 3.14: Topographical distribution of significant clusters associated with a dependent samples t-test for theta activity (3 – 7 Hz) between the theta and beta sound conditions of the Moderate MC group during the study phase. Theta band power differed between the theta and beta sound conditions in frontal sensors. The color bar represents T-values of the t-test for each topographical plot (yellow represents a higher T-value and blue represents a lower T-value). Significant channels ($p < 0.05$) are marked with (*),

The increase in theta power in the theta condition compared to the beta condition within the Moderate MC condition indicates that the theta sounds were impacting neural activity. No clusters were found when comparing sound condition within the No MC and Mild MC groups. Since noisy channels may have been affecting clustering, we averaged over the 8 frontal-central electrodes (F3, FC1, Cz, FC2, F4, F1, F2, FC4) to determine if there

were differences in frontal-midline theta specifically (Supplementary Figures 1 & 2). When averaging across these electrodes, dependent-samples t-tests with Monte Carlo correction between sound conditions in each MC showed some differences (Supplementary Tables 1 & 2). However, since the original ANOVA did not show a main effect of sound condition, these differences will not be extensively explored here.

Chapter 4: Discussion

The goals of this study were to: 1) examine the effect of auditory stimuli at an individual's preferred theta frequency on EEG theta power, and 2) determine if objects paired with theta stimulation were remembered better. Per the first hypothesis, we did find a trend toward increased theta power in frontal electrodes while an individual's preferred theta frequency was playing (Figure 3.8). However, theta stimulation was not necessarily linked with enhanced memory for object locations across participants as we predicted in our second hypothesis. In an effort to explore why this was, we grouped the data into 3 groups according to participants' Subjective Memory Complaint Scores. Several differences were found between Memory Complaint groups, indicating the effects of auditory theta stimulation are not uniform in older populations. Thus, this discussion will focus on factors that influenced individual's memory performance, including and in addition to auditory stimulation, and how to refine these methods to identify vulnerable populations and optimally impact memory.

4.1 Preferred Theta

The first key finding of this study was that individuals aged over 50 preferred different theta frequencies within the 3 – 7 Hz band. This is the first time preferred theta frequencies have been measured, although there is extensive literature on peak alpha frequencies.[224–227] Much like peak alpha, an individual's preferred theta frequency can be correlated with an individual's cognitive performance. In this study, individuals with a preferred theta frequency of 6 Hz exhibited better memory scores than individuals who preferred 4 Hz, regardless of their subjective memory complaint scores. Larger group sizes for all of the frequencies within the theta band would help elucidate memory changes associated with preferred theta frequencies going forward.

4.2 Gender Differences

An unforeseen finding was the deficit in memory for theta condition object-locations in females compared to males, despite similar age and memory group distributions. This difference is not necessarily surprising given the predominance of Alzheimer's and dementia in women, however, it is surprising to see such a large difference in a 'healthy' middle-aged cohort.[228,229] Small sample size and unequal group sizes could be contributing to this finding, but, it could indicate other underlying mechanisms impacting memory like menopause and estrogen decreases.[230,231] A significant interaction between the APOE4 allele, the major genetic risk factor for "sporadic" forms of AD, and gender has also been found.[232,233] APOE4 female carriers display significantly decreased DMN connectivity compared with men of the same genotype and non-carrier females.[234] Notably this was evident preceding any cognitive impairment. As mentioned in the introduction, the DMN overlaps with memory circuitry, including the theta circuit in the hippocampus and the Papez Circuit. A future study could test for APOE genotype and use MEG/EEG to more thoroughly explore the gender differences in memory circuitry in association with AD risk genes.

4.3 Beta Activity – A Future Target

Another unexpected finding was the increase in high beta activity (20 – 30Hz) seen in the No MC group during the study phase. This is especially interesting since this increase in beta activity occurred in the theta condition while theta sounds were playing (not beta sounds). This increase in beta activity could be due to harmonics of the preferred theta frequency, or it is also possible that tuning the sensory stimuli to the brain's preferred theta frequency could put the brain in a more optimal state to promote cross-frequency coupling. Mouse studies also indicate high beta (23 – 30 Hz) in a brain state that promotes new learning that decreases once information is learned.[235] This could explain why the No MC Group had more high beta activity during the study phase when they were first learning the object-locations compared to

the learning phase when they were practicing what they had already learned. The absence of this high beta activity in the other two memory groups during the study phase could be reflective of a suboptimal brain state for new learning. Decreases in beta activity have been associated with early stages of cognitive decline as well.[236]

In contrast to the study phase, the Mild MC and Moderate MC Groups both exhibited more beta activity in the theta condition compared to the No MC group. It is important to note that the increase in beta activity for the Mild and Moderate MC groups during the learning phase was in the lower beta band (13 – 20 Hz) which is associated more with attentional resources and voluntary motor planning, which we would expect to see during the learning phase.[235,237–239]

The within group analyses shed some more light on this increase in beta activity. Our initial hypothesis was that the beta condition would have more beta activity due to the beta auditory stimulation. Yet, compared to the beta condition in the study phase, the theta condition still displayed more high beta activity (23 – 30 Hz). It is important to note that every participant received 15 Hz beta stimulation which may not have been an optimal frequency for each person, and could have even been detrimental to the ongoing beta rhythm. Therefore, by putting the brain in a more optimal state, preferred theta frequency auditory stimulation may have inadvertently promoted beta oscillations, while beta auditory stimulation at 15 Hz failed to stimulate beta activity. This hypothesis is further supported by Supplementary Figures 1 & 2 that display difference plots (theta – pure) and (theta – beta), which look quite similar (and not statistically significant), indicating that beta stimulation at 15 Hz was more or less equivalent to stimulation with pure pink noise. These findings point towards the inter-individual variability in brain rhythms and the importance of evaluating an individual's natural brain rhythms prior to neurostimulation.

It is still up for debate whether certain frequencies within the theta band are 'better' or 'worse' than others. If it is overall theta band power that is most important to cognition, then stimulating at an individual's preferred theta frequency would be most effective. However, if the oscillatory slowing associated with aging is the primary factor in cognitive decline (not decrease in oscillatory power), then attempting to stimulate activity at more 'beneficial' frequencies could be an option, but the findings of this study suggest that individuals may not entrain as well to frequencies outside of their preferred range.

4.4 Subjective Memory Complaints

One of the most significant findings of this study was that participants realistically sorted themselves into No MC, Mild MC, and Moderate MC groups. This was reflected in significantly lower MoCA and memory scores for the Moderate MC group compared to the other groups. However, it is important to note that all participants had a MoCA greater than 24, suggesting there were no cognitive impairments (sensitive to the test), including in the Moderate MC group. More importantly, there was also a difference in their neural activity, indicating deeper underlying mechanisms of memory impairment. The MC groups did not significantly differ in age (M = 62 years), exhibiting how early these neurological changes can occur alongside mild to moderate memory complaints.

Behaviourally, we expected individuals in the Moderate MC Group to perform more poorly on the memory task, needing more memory trials and displaying worse memory scores. Interestingly, there was no difference in the number of learning trials between memory groups, but within the Moderate MC group, significantly more trials were needed to learn object-locations in the beta condition compared to the theta condition. This could be explained by the significant increase in EEG theta activity during the theta sound condition compared to the beta sound condition during the study phase. Increased theta activity has

been associated with enhanced encoding, which could have led to fewer learning trials for the correctly encoded theta objects.[240–242]

However, this EEG theta increase for the theta condition disappears during the learning phase and the Moderate MC group went on to display overall lower memory scores compared to the other MC Groups, particularly in the theta condition. Although the objects in the theta condition may have been encoded, the fewer learning trials may have led to retroactive interference, with the more recently learned objects with more trials interfering with the memory for theta objects.[243–245] Therefore, the auditory theta stimulation may be most beneficial when applied during encoding rather than learning. High theta activity has also been associated with the receipt of negative feedback, thus, increasing theta activity during learning may not always be beneficial.[246] It is possible that by stimulating theta activity with theta sounds during learning, we may have destabilized memories for object locations that had been correctly encoded. In a future study on the effects of auditory stimulation specifically on learning, it would be interesting to withhold auditory stimulation during the first learning trial, then pair correctly recalled objects with beta activity to reinforce correct memories,[246,247] and pair incorrectly recalled objects with theta activity to encourage the modification of the memory.[248]

Conversely, the Mild MC group displayed significantly better memory scores in the theta sound condition compared to the moderate MC theta sound condition. Since there was no difference in memory at the first learning trial, this difference must have emerged during feedback learning. Lower EEG theta activity during the learning phase may have actually helped the Mild MC group and it is possible they were not as susceptible to age-related increases in interference as the Moderate MC group.[249]

Our findings agree with several other studies that have found a relationship between subjective memory complaints and memory performance. Additionally, the differences in

EEG theta, alpha, and beta activity between MC groups are novel contributions to this literature. Not only does this finding reaffirm that subjective memory complaints should be taken seriously by researchers and physicians, but it is also indicative of early memory circuit dysfunction.[250] Previous studies have shown individuals with subjective memory complaints display smaller left hippocampal and EC volumes as well as temporal white matter loss.[251–254] Decreased hippocampal and EC volume could underly the differences in theta activity found between MC groups. Furthermore, APOE4 carriers with subjective memory complaints showed higher CSF phospho-Tau and phospho-Tau/Aβ42 levels compared to APOE4 carriers without memory complaints and normal APOE4 negative controls.[255–257] A future study integrating all of these criterion by measuring memory complaints and memory performance in relation to EEG activity, CSF phospho-tau and phopsho-tau/Aβ42 levels, as well as hippocampal and EC volume would provide the most complete picture of how subjective memory complaints relate to memory circuitry.

4.5 Conclusions and Future Directions

Overall, this study indicates that electrophysiological differences between individuals with no memory complaints, mild memory complaints, and moderate memory complaints are reflective of underlying circuit dysfunction that could be caused by age-related changes in the brain. The molecular changes mentioned in the introduction associated with aging and, in pathological cases, Alzheimer's Disease directly and indirectly influence the expression of LTP and LTD at synapses, impacting memory and synaptic plasticity. In this study, we investigated the summation of all of these small synaptic alterations based on their influence on local field potentials (LFPs) with EEG recordings.[17,258,259]

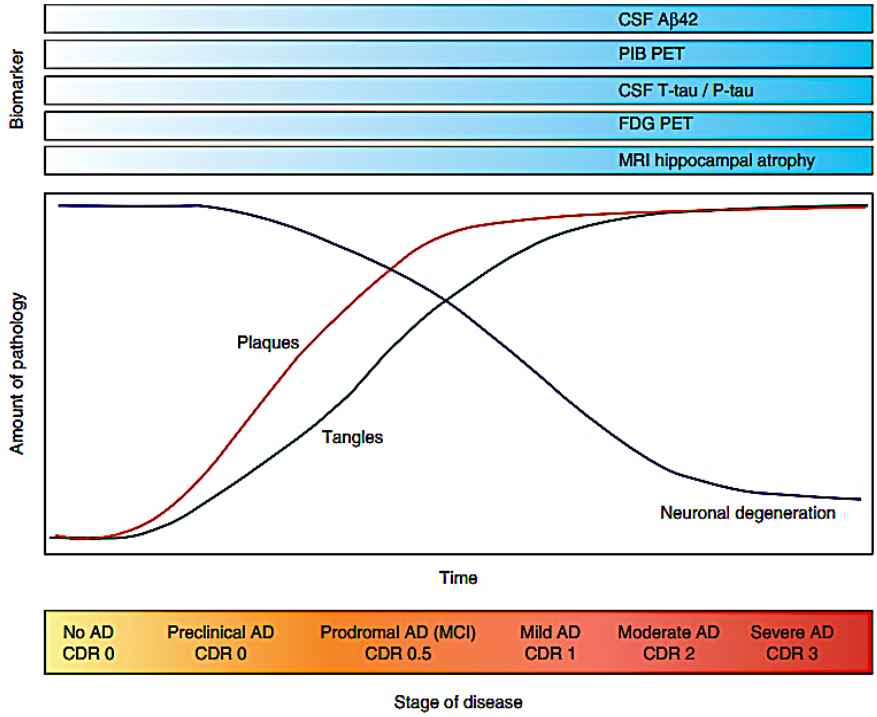

Figure 4.1: Figure from Blennow et al. 2012 depicting how increases in plaques and tangles occur in tandem with neurodegeneration. The top of the figure also shows how different biomarkers for AD increase over time in association with the AD staging listed at the bottom.

As seen in Figure 4.1 from Blennow et al. 2012, there is an established connection between pathological changes, AD staging, and neuronal degeneration.[260] However, electrophysiological changes are noticeably absent. The most complete understanding of AD can only be achieved when we take all of the neurological changes into consideration—from individuals molecules, to electrical circuitry, to structure, all the way up to memory and subjective experience. The current study is a first step toward providing this missing link between the electrophysiology and memory changes that occur in conjunction with changes in neural pathology and structure over time. We already found stark differences in neural activity in individuals with subjective memory complaints beginning as early as 50 years old. Next, there needs to be a systematic evaluation of electrophysiological changes associated with normal aging vs AD in multiple age groups to determine exactly where the groups diverge and if there is a point in time when pathological changes can be reversed or prevented from progressing. EEG is a valuable tool for this continuous assessment in its ability to track changes with high time resolution.

Finally, this study contributes to the accumulating evidence of the validity of using non-invasive sensory stimulation to beneficially impact neural activity.[261–263] Although the focus of most of this thesis has been on bridging the gap from cellular components upward toward the systems level, the signalling systems in the brain do not operate in just one direction. When we alter the synchronization of different neural circuits with auditory stimulation, we are also influencing the cellular components that underly these circuits. Pharmaceuticals frequently target one molecule and fail to control multifaceted diseases like AD; yet, something as simple as listening to sounds at specific frequencies could improve brain health at multiple levels simultaneously and help our aging population retain their memories.

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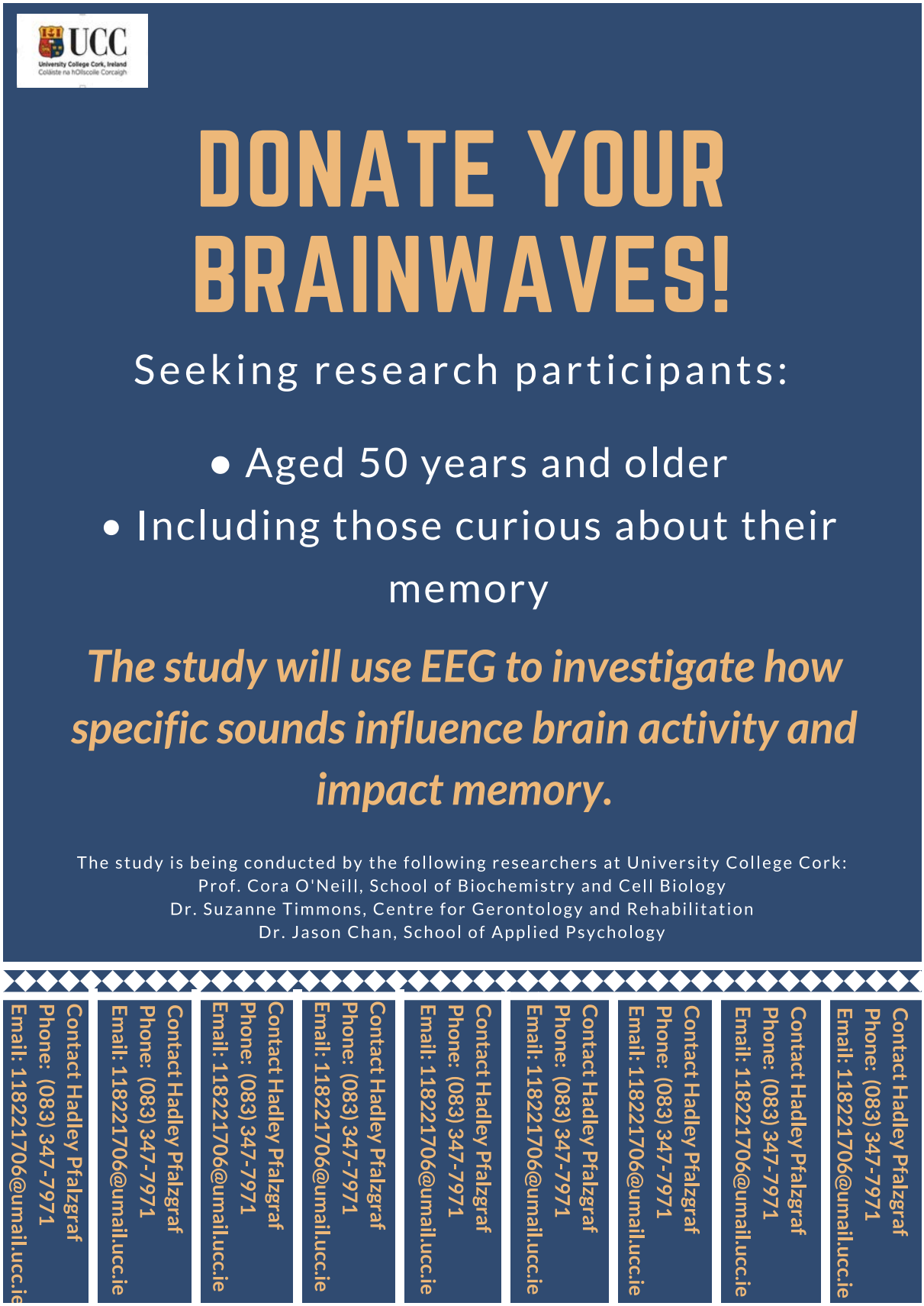
Appendix i: Study Advertisement

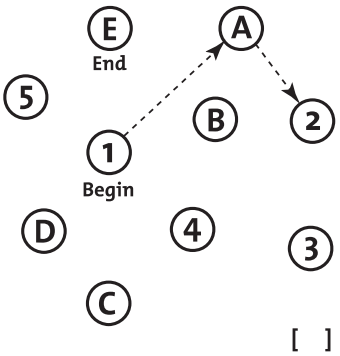


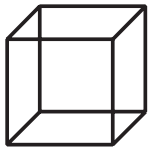
Appendix ii: Montreal Cognitive Assessment

MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS



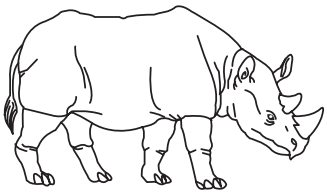


Copy cube [ ] [ ]

Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands __/5

NAMING

[ ]



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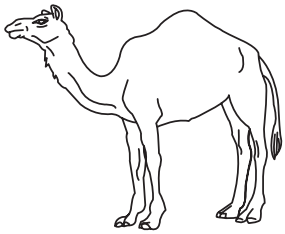


[ ] __/3

MEMORY Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 __/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** __/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] __/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words) __/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler __/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only
		[ ]	[ ]	[ ]	[ ]	[ ]	
Optional	Category cue						
	Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City __/6

 Version November 7, 2004
www.mocatest.org

Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Appendix iii: Subjective Memory Complaint Questionnaire

Dement Neuropsychol 2012 December;6(4):212-218

APPENDIX

MCS - MEMORY COMPLAINT SCALE

VERSION A - PATIENT ANSWERS

Objective: To assess patient's memory complaint directly with him/her

Instructions:
- Apply this directly to patient with no intervention from companion
- Read aloud in a clear voice

Q1. Do you have any memory problems? (or "forgetfulness?" or "memory difficulties")

( ) No = 0 ( ) Unable to answer/unsure/doubt = 1 ( ) Yes = 2

If answers No, mark 0 and likewise for Q2 and Q3 and skip ahead to Q4

Q2. How often does this happen?

( ) Rarely = 0 ( ) Occasionally/sometimes =1 ( ) A lot/frequently = 2

Q3. Does this memory problem hamper (or impair) your daily activities?

( ) No = 0 ( ) Occasionally/sometimes = 1 ( ) A lot /frequently = 2

Q4. How is your memory compared to others your age?

( ) The same or better = 0 ( ) Somewhat worse = 1 ( ) Much worse = 2

Q5. How is your memory compared with when you were younger?

( ) Same or better = 0 ( ) Somewhat worse = 1 ( ) Much worse = 2

Q6. Do you forget what you've just read or heard (e.g., in a conversation)?

( ) Rarely/never = 0 ( ) Occasionally = 1 ( ) Often = 2

Q7. Rate your memory on a scale of 1 to 10, with 1 worst and 10 best

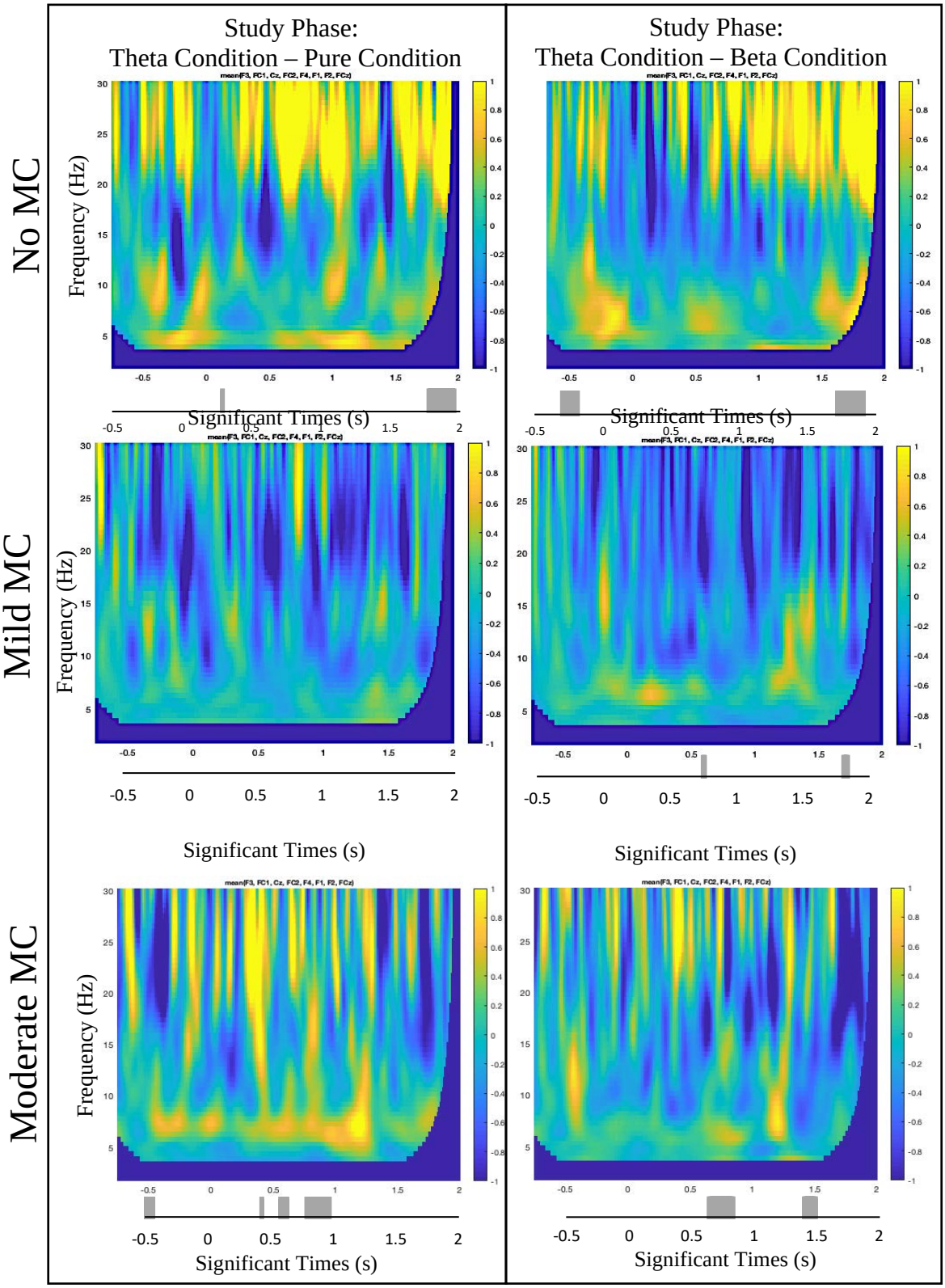
( ) 9 or 10 = 0 ( ) 5 to 8 = 1 ( ) 1 to 4 = 2

Scoring

Interpretation

[ ] No MCs (0-2) [ ] Mild MCs (3-6) [ ] Moderate MCs (7-10) [ ] Severe MCs (11-14)

Supplementary Figures



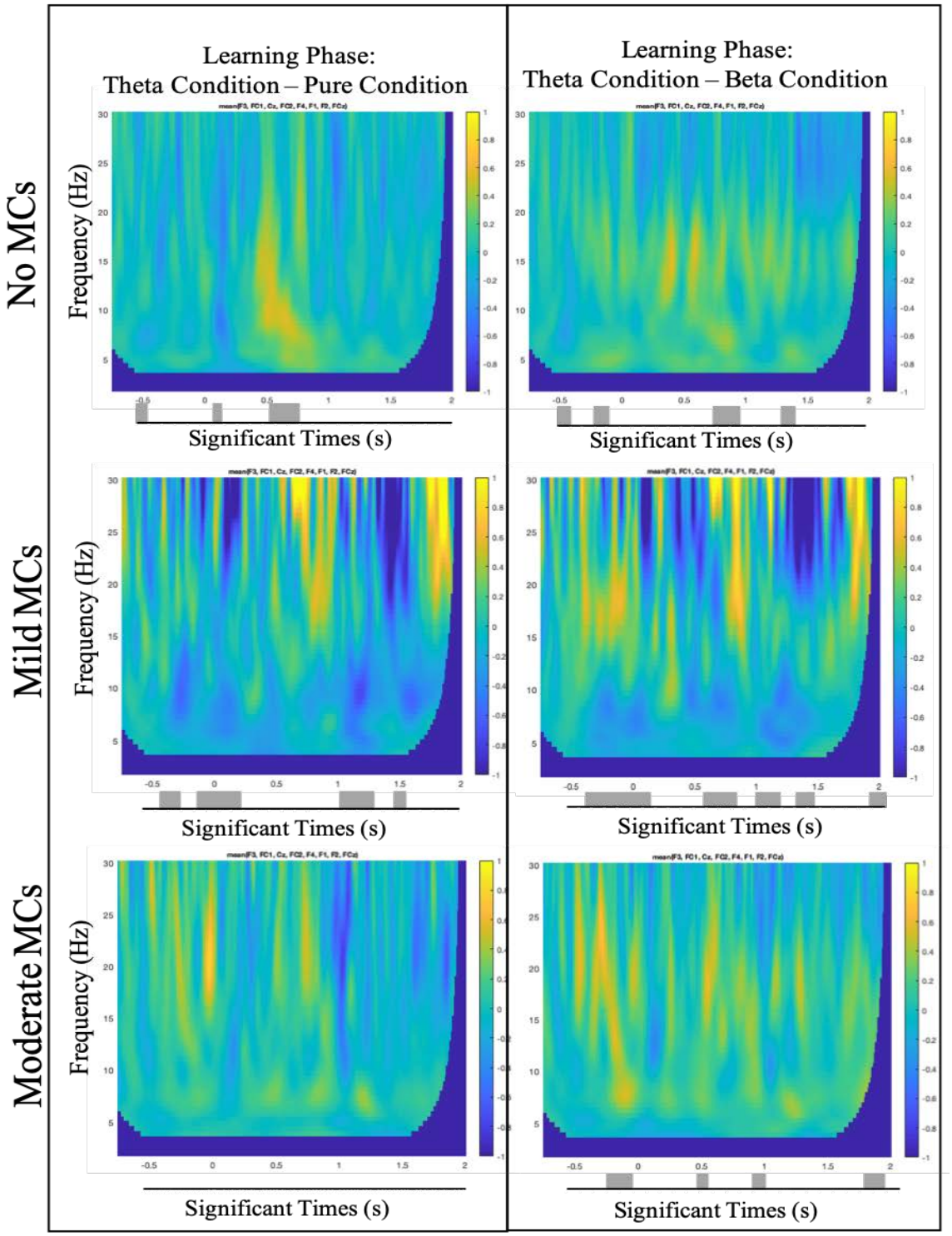
Supplementary Figure 1: Time frequency spectral power differences between sound conditions—left column (Theta – Pure), right column (Theta – Beta)—for each MC group during the study phase. Times associated with significant differences in theta (3-7 Hz) power are marked with grey boxes below the x-axis (P <0.05). ***Top****) No MC difference plots, color bar represents z-scores of power values (yellow indicates higher power in the theta sound condition, dark blue indicates higher power in the pure sound condition (left) or beta sound condition (right)).* ***Middle****) Mild MC sound condition difference plots.* ***Bottom****) Moderate MC sound condition difference plots. This plot has the largest increase in theta power in the theta condition compared to the pure condition.*

No MC Study T-tests	Theta x Pure Condition	Theta x Beta Condition
Times with Significant Differences in Theta Power (s)	1.78 – 1.96	-0.3 to -0.19; 1.72 – 1.91
Significant Frequencies Averaged over time (Hz)	7.0 – 10.0	23.0 – 30. 0

Mild MC Study T-tests	Theta x Pure Condition	Theta x Beta Condition
Times with Significant Differences in Theta Power (s)	None	0.74 – 0.76; 1.81 – 1.85
Significant Frequencies Averaged over time (Hz)	16.0 – 24.0	22.0 – 30.0

Moderate MC Study T-tests	Theta x Pure Condition	Theta x Beta Condition
Times with Significant Differences in Theta Power (s)	-0.5 to -0.44; 0.42 – 0.44; 0.58 - 0.63; 0.79 – 1.0	0.64 – 0.83; 1.39 – 1.5
Significant Frequencies Averaged over time (Hz)	7	15.0 – 30.0

Supplementary Table 1: T-tests between sound conditions (left: Theta x Pure; right: Theta x Beta) during the study phase for the three MC groups. The first row in each table reiterates the time points when theta activity significantly differed between sound condition indicated with grey bars in supplementary figure 1 ($P < 0.05$). The second row is the result of averaging over time to examine which frequencies besides the theta band differed between sound conditions ($P < 0.05$). The theta condition in the No MC group exhibited significantly more alpha and high beta (23 – 30 Hz) activity compared to the pure and beta conditions respectively ($P < 0.05$). The theta condition of the Mild MC group also exhibited differences in beta power (16 – 30 Hz) compared to the pure and beta conditions. The theta condition of the Moderate MC group displayed higher theta activity (7 Hz) compared to the pure condition, and also showed a differences in beta activity in the beta condition like the other MC groups.



Supplementary Figure 2: Time frequency spectral power differences between sound conditions—left column (Theta – Pure), right column (Theta – Beta)—for each MC group during the learning phase. Times associated with significant differences in theta (3- 7 Hz) power are marked with grey boxes below the x-axis (P <0.05). ***Top****) No MC difference plots, color bar represents z-scores of power values (yellow indicates higher power in the theta sound condition, dark blue indicates higher power in the pure sound condition (left) or beta sound condition (right)).* ***Middle****) Mild MC sound condition difference plots.* ***Bottom****) Moderate MC sound condition difference plots. This plot has the largest increase in theta power in the theta condition compared to the pure condition.*

No MC Learn T-tests	Theta x Pure Condition	Theta x Beta Condition
Times with Significant Differences in Theta Power (s)	-0.5 to -0.42; 0.12 – 0.15; 0.57 – 0.77	-0.5 to -0.42; -0.19 to -0.11; 0.78 – 0.96; 1.33 – 1.41
Significant Frequencies Averaged over time (Hz)	None	None

Mild MC Learn T-tests	Theta x Pure Condition	Theta x Beta Condition
Times with Significant Differences in Theta Power (s)	-0.35 to -0.22; -0.6 - -0.26; 1.07 – 1.32; 1.5 – 1.57	-0.34 – 0.14; 0.58 – 0.82; 1.0 – 1.17; 1.31 – 1.43; 1.9 – 2.0
Significant Frequencies Averaged over time (Hz)	4.0 - 10.0	5.0 – 8.0

Moderate MC Learn T-tests	Theta x Pure Condition	Theta x Beta Condition
Times with Significant Differences in Theta Power (s)	None	-0.19 to – 0.03; 0.49 – 0.54; 0.90 – 0.97; 1.75 – 1.87
Significant Frequencies Averaged over time (Hz)	None	6.0 – 7.0; 14.0 – 17.0

Supplementary Table 2: T-tests between sound conditions (left: Theta x Pure; right: Theta x Beta) during the learning phase for the three MC groups. The first row in each table reiterates the time points when theta activity significantly differed between sound condition indicated with grey bars in supplementary figure 2 ($P < 0.05$). The second row is the result of averaging over time to examine which frequencies besides the theta band differed between sound conditions ($P < 0.05$). The theta condition in the No MC group did not significantly differ at any frequencies from the other conditions. The theta condition of the Mild MC group exhibited differences restricted to the theta band compared to the pure and beta conditions ($P < 0.05$). The theta condition of the Moderate MC group only differed from the beta condition with higher power in the theta band (6 – 7 Hz) and low beta band (14 – 17 Hz).