Title:

Resting Motor Threshold – Towards a TMS biomarker of Cognitive Decline in Alzheimer's Disease

Subtitle:

Investigating cortical excitability & cognition in people with dementia

Student: Siddhesh Zadey

Registration No.: 20131079

Department: Division of Biology

Institution: Indian Institute of Science Education & Research (IISER), Pune

Research Mentor: Dr. Alvaro Pascual-Leone

Director, Berenson Allen - Center for Non-invasive Brain Stimulation

(BA - CNBS)

Department Head, Cognitive Neurology, Beth Israel Deaconess Medical Center

(BIDMC)

Dean, Translational & Clinical Research, Harvard Medical School (HMS)

Certificate:

This is to certify that this dissertation titled *Towards a TMS biomarker of cognitive decline in Alzheimer's Disease* towards the partial fulfilment of the
BS-MS dual degree programme at the Indian Institute of Science Education and Research,
Pune represents study/work carried out by *Siddhesh Zadey at the Berenson-Allen Center for Non-invasive Brain Stimulation (BA-CNBS), Harvard Medical School, Boston, MA*, under the supervision of *Dr. Alvaro Pascual-Leone, Director BA-CNBS,* during the academic year 2017-2018.

Signature

Student's name: Siddhesh Zadey

Date: 15th March 2018

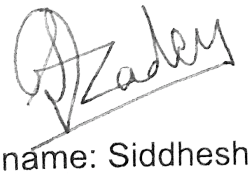
Signature

Mentor's name: Dr. Alvaro Pascual-Leone

Date: 15th March 2018

Declaration:

I hereby declare that the matter embodied in the report titled *Towards a TMS biomarker of cognitive decline in Alzheimer's Disease* are the results of the work carried out by me at the *Berenson-Allen Center for Non-invasive Brain Stimulation (BA-CNBS), Harvard Medical School, Boston, MA* under the supervision of *Dr. Alvaro Pascual-Leone, Director BA-CNBS* and the same has not been submitted elsewhere for any other degree.



Signature

Student's name: Siddhesh Zadey

Date: 15th March 2018

Signature

Mentor's name: Dr. Alvaro Pascual-Leone

Date: 15th March 2018

Dedication

In the loving memory of my first and oldest friend, my grandfather – Vinayak Paralikar, who taught me that learning is the breath of life.
I hope this work makes his peaceful soul proud.

Acknowledgments:

First and foremost, I would like to express my most profound gratitude towards Dr. Alvaro Pascual-Leone for hosting me at the Berenson-Allen Center for Noninvasive Brain Stimulation (BA-CNBS) & agreeing to mentor this thesis. In addition to providing resources (intellectual and otherwise) that were vital for this thesis, I would like to thank Dr. Pascual-Leone for providing me an opportunity to imbibe his thought (or hypothesis)- driven research practice & undying curiosity for newer problems. I would also like to thank Dr. Collins Assisi for being a member of the thesis advisory committee for this project. The ingenious discussion provided by him through the mid-term has made a significant dent in the course of the project in the past few months. I extend my gratitude to Dr. Peter Fried, who has been an acting co-mentor for a large part of this thesis & my work at BA-CNBS. This thesis would not have been completed without my partners in crime - Dr. Stephanie Buss, Katherine McDonald & Arianna Menardi. I thank all the members of the BA-CNBS for making this a truly stimulating year, with a particular mention of Alisha & Andrea for making my visit possible. I am obliged to Dr. Aseem Ansari and other members of the Khorana Program along with Mr. Avi Nash, who made my year-long stay not only possible but also comfortable.

Over the years, several mentors have had an insurmountable impact on my thinking that is inseparable from my work. As a representation of my gratitude to all of them, I would like to thank Dr. Edward Hubbard, Dr. Nishikant Subhedar, Dr. Semir Zeki, Prof. Sameer Phale, Dr. C. S. Yajnik and more recently, Dr. Abhay Bang & Dr. Krish Sathiyan.

This year has been somewhat challenging but hugely rewarding for me. I can never be grateful enough to my family (beyond the one with which I share my genes) that has helped me through all the difficult times. I am in debt to my parents (Dr. Varsha & Dr. Gajanan Zadey) for bringing me into existence and tolerating it (my existence) thereafter. I (have to) thank (now) Dr. Sweta Dubey for being a core member of this *legion of tolerance* for the past four years now. Finally, I would like to thank my reliable support - my friends & cousins, who are understanding enough to know that I cannot name all them here.

Contents:

Abstract:

The growing burden of dementia due to Alzheimer's Disease (AD) across the globe is currently one of the primary geriatric health concerns. The past decades have observed inspiring progress in understanding the fundamental neuropathological changes contributing to AD causation and progression. Several genetic, physiological & neuroimaging biomarkers have been identified for predicting the diagnosis, monitoring the prognosis and evaluating the treatments. The multifactorial nature of the disease necessitates markers that capture specific aspects of the brain pathology and serve different functions. The primary premise of this thesis is the proposal of a novel marker of cognitive decline in AD. Transcranial magnetic stimulation (TMS) is a technique employed for non-invasive measurement of the cortical excitability in humans. A specific contributor of the total cortical excitability is the excitability of the axonal membranes in the neuronal systems, which in the case of the motor cortex, can be measured by the TMS resting motor threshold (RMT). We present an evaluation of RMT as an independent neurophysiological marker of the cognitive decline in the AD, analyzed across multiple cohorts (group comparison cohort, multicentre disease cohort & group of past studies) in diverse experimental settings (research facilitation and clinical setting) by multiplex statistical strategies (extensive cross-sectional correlational analyses & meta-analyses). While the notion of cortical hyperexcitability in the AD is prevalent, one of the significant contributions of the thesis is an original biological rationale specific for the abnormal RMT in AD compared to healthy aging. The thesis further extends to test the effect size of the RMT abnormality in a broader quantitative synthesis of published studies.

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Introduction to Discussion page count = 48

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1. Introduction:

Dementia due to Alzheimer's Disease (AD) presently affects 46.8 million people worldwide. More alarming than the current prevalence is the incidence, which estimates that a new case of dementia is reported every three seconds. Of these, about 58% people with dementia would be citizens of low-and-middle-income countries (LMIC) including India. Approximately 4.1 million Indians are afflicted with the AD and the forecasted growth in for this number for the region over the next 12 years is around 82%. Taking into account the increasing population size of the Indian sub-continent along with near exponential rise in the life expectancy over the past decades, a growth of this magnitude in dementia cases will put a burden of more than $12 billion (~ 7680 crores INR; according to current valuation) on our healthcare system by 2030 (World Alzheimer Report, 2015). Hence, active measures across research and clinical care are requisite for tackling AD.

AD is marked by the abnormal/accelerated decline of cognitive function with progressing age (Figure 1.1). While initial, mild stage of AD starts primarily with memory impairments, the moderate stage is marked by failure in other cognitive domains, including attention, orientation, language speaking, comprehension, etc. leading to failure of global cognition. In the later, severe stage, there might be deterioration of motor system as well. This progressive loss of cognition over time affects a person’s day-to-day activities and results in an overall depreciation of quality of life, ultimately leading to a stage of handicap & complete dependency on external care.

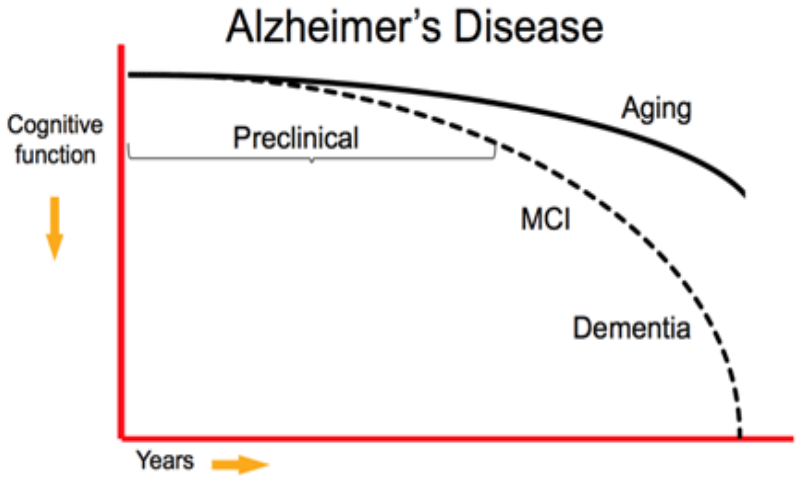

Figure 1.1: Theoretical model of abnormal / accelerated aging in AD: Compared to healthy aging, ,AD portrays a rapidly progressive decline in cognitive function through symptomatically pre-clinical stage (marking short-term memory loss) & mild cognitive impairment (MCI – marked by rise in the impairment of global cognition), ultimately resulting in Dementia. Figure adapted from (Sperling et al., 2011).

1.1 A neurobiological background of Alzheimer's Disease

AD is a neurodegenerative disorder where neuropathological developments result in progressive synaptic dysfunction, loss of synapses & neuronal death. The multifactorial nature of the underlying neuropathology AD makes it a curious research problem and a complex issue from the perspective of clinical management. Several complementary and competing hypotheses have been presented and tested in the past three decades that attempt to explain the cause as well as the mechanisms of pathological changes in the brain evolving over the course of AD progression. For instance, the most comprehensive Aβ (Amyloid beta) hypothesis explicates that the increased accumulation amyloid oligomers & plaques through a cascade of downstream events – microglial activation, oxidative injury, impairment of ion homeostasis, formation of neurofibrillary tangles, etc. result in synaptic & neuretic injury, which is reflected in the cognitive dysfunction (Selkoe and Hardy, 2016). While the growing evidence for Tau hypothesis presents the notion that increased amount of hyperphosphorylated Tau peptide & deposition of neurofibrillary tangles (NFT) formed by Tau could be primary agent triggering the cascade that leads to neurodegeneration (Maccioni et al., 2010). Involvement of neuroinflammation involving glial activation, neurotoxicity due to building up of metallic ions, oxidative &

metabolic stress effects, have complemented these hypotheses in understanding the events leading to neurodegeneration (Mohandas et al., 2009).

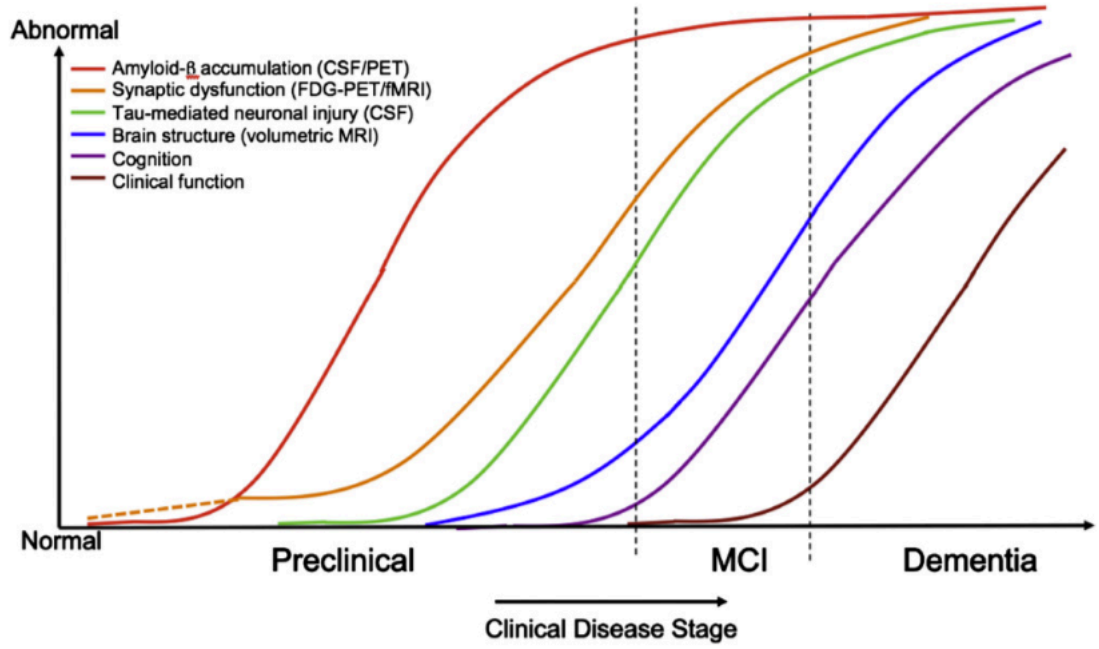

Figure 1.2: The chronobiological model of dynamic biomarkers of Alzheimer's Disease: The model here depicts the nature of changes in the levels of the biomarkers over from normal to abnormal over an extended period, marker by disease stages. The horizontal distance between the points at the same height on the two curves mark the magnitude of correlation – greater the distance, lesser the correlation. Hence, Amyloid or FDG-PET are useful biomarkers for AD incidence while volumetric MRI is a better marker for cognitive decline. Further a steeper slope of the linear part represents faster neuropathology development. Figure adapted from (Sperling et al., 2011).

While the research on the probable components that explain the AD pathology continues to grow, we have been able to identify certain key factors that can be of utility for 'marking' the AD pathology and neurodegeneration. A gamut of longitudinal multicohort multimodal studies has contributed to the development of dynamic AD biomarkers. These biomarkers are compiled in chronobiological models to understand AD progression (Jack Jr et al., 2010). A marker that has a greater correlation with the cognitive dysfunction is considered to have evolved later in the temporal course of AD progression. Such markers reflect downstream events in the pathology cascade (Jack, 2011). For example, brain atrophy, reflective of neuronal (and synaptic) loss, as measured by Magnetic Resonance Imaging (MRI) is a 'late' neurodegeneration biomarker (Lo, 2011). While a marker with smaller (or no) correlation with cognitive decline is thought to have evolved earlier in the time & have

a causal role in the evolution of pathology. For instance, abnormal Aβ-42 (a subtype of the amyloid peptide) levels in cerebrospinal fluid (CSF) or Aβ deposits measured by Amyloid – Positron Imaging Tomography (PET) are considered ‘early’markers. Hence, these models are useful for understanding the temporal sequence of pathological events & for drawing plausible inferences about causality between these events. Addition of new biomarkers that explore novel facets of the AD pathology is an important quest having implications on research and clinic; from diagnosis to treatment.

One possible biomarker of AD could be the altered excitability in neuronal circuits reported across computational, animal-model & human research. In the case of translational research involving humans, transcranial magnetic stimulation (TMS) is becoming increasingly pivoting to understand the mechanisms and the consequences of hyperexcitable AD cortex. Previously, TMS studies, employing specific paradigms/protocols, have investigated the involvement of disruption of cholinergic pathways, glutamatergic excitotoxicity & reduction in GABA (Gamma-Aminobutyric Acid)-mediated disinhibition to infer the underlying principles of cortical hyperexcitability (Freitas et al., 2011).These TMS protocols have been utilized for two inter-related purposes – for deciphering the mechanisms of hyperexcitability & for measuring the efficacy of available treatment methods. The initial support in the literature was skewed to some extent towards the notion that deficit of acetylcholine could be most dominant candidate driving hyperexcitability. However, the inadequate success of acetylcholinesterase inhibitors (AChEIs) as a treatment method for preserving cognitive function has questioned the credibility of the cholinergic hypothesis. The support for glutamatergic excitotoxicity as a treatment candidate, through interventions involving NMDAR (N-methyl D-aspartate receptors) antagonists,is being actively evaluated with equivocal results (Di Lazzarro et al., 2003). While TMS studies (in corroboration with EEG & fMRI, pharmacological studies) have shown impairment in GABA-ergic circuits, the understanding of biomarker utility & treatment targets is still in naïve stages. This necessitates continuing the exploration of cortical hyperexcitability biomarkers that are grounded in biological knowledge of AD neuropathology, can explicate (or at least correlate to) the cognitive decline & provide notions for novel treatment targets.

1.2 Understanding TMS Resting Motor Threshold

Transcranial magnetic stimulation (TMS) is a technique that performs stimulation of cortical brain regions in a non-invasive manner. Theoretically founded on Faraday's laws of electromagnetic induction, the fundamental notion of TMS is to induce electric discharge in neuronal element situated proximally to the TMS coil conducting time-varying current produced by the magnetic stimulator. This provides a unique opportunity for measurement as well as manipulation of the neuronal currents in superficial cortical regions. Resting motor threshold (RMT) is a TMS protocol in which low frequency consecutive single pulses are delivered to the region of interest (corresponding to the target muscle) in the motor cortex, and the effect of stimulation is judged based on the generated motor response called motor evoked potential (MEP). Operationally, RMT is defined as the minimum intensity of stimulation measured as, % maximum stimulator output (% MSO), that is required to garner an MEP of peak-to-peak amplitude ≥ 50 μV in at least 5 out of 10 trials. This method of RMT measurement called the relative frequency method is the most common method practiced across TMS centers (Neuromethods, Tanscranial Magnetic Stimulation, Springer).

RMT is considered to measure the baseline cortical excitability in a person and is also thought to be reflective of their corticospinal integrity (Battaglia et al., 2007). RMT depends on several factors that can be classified as physiological (person-dependent) & physical (stimulation-dependent) (Delvendahl et al., 2014). The person-dependent factors include –
age – decrease in excitability with healthy aging (Rossini et al., 2007), gender – changes due to hormones such as progesterone, cortical thickness – determines the target at the depth of stimulation (Danner et al., 2012), orientation of underlying white matter fibre bundles (Amassian et al., 1992) (Herbsman et al., 2009)– determines differential electric discharge, scalp-to-cortex distance – determines the extent of current reaching the brain after attenuation due to CSF & other layers, genetic factors, eg: BDNF (Brain Derived Neurotrophic Factor) – allelic polymorphism

changes synaptic plasticity (Teter and Ashford, 2002). The stimulation-dependent factors include –
intensity of stimulation (determines the amplitude of current delivered at the cortex), phase (determines changes in latency), coil shape (determines the distribution of underlying electric field, coil size (determines focality and depth of electric discharge), position (targeted neuronal region), direction/orientation (determines the direction of current flow in the brain & hence which neuronal populations will be excited) (Nagarajan et al., 1993)(Sommer et al., 2006). While this is not an exhaustive list of determinants of RMT, these are some factors that may lead to variability in measurements.

1.3 The *primum ratio* of this thesis

Depending on the coil position & orientation, the recruitment of the underlying neurons along the corticospinal tract (CST) that are involved in eliciting the MEP changes. From epidural recordings, animal nerve models & computational approximations, the general notion is that RMT involves either the direct activation of corticospinal neurons (layer 5) or their trans-synaptic activation through the excitation of the axonal fibers of the cortico-cortical neurons (interneurons) (layer 2-3)(Triesch et al., 2015). Hence, while the target specifications might change, the cotribution of the excitation of neuronal membrane towards RMT measurement is undeniable. These cortical activations are transmitted through the spinal projection in the form of descending volleys of current that finally excite the target muscle response, measured as MEPs.

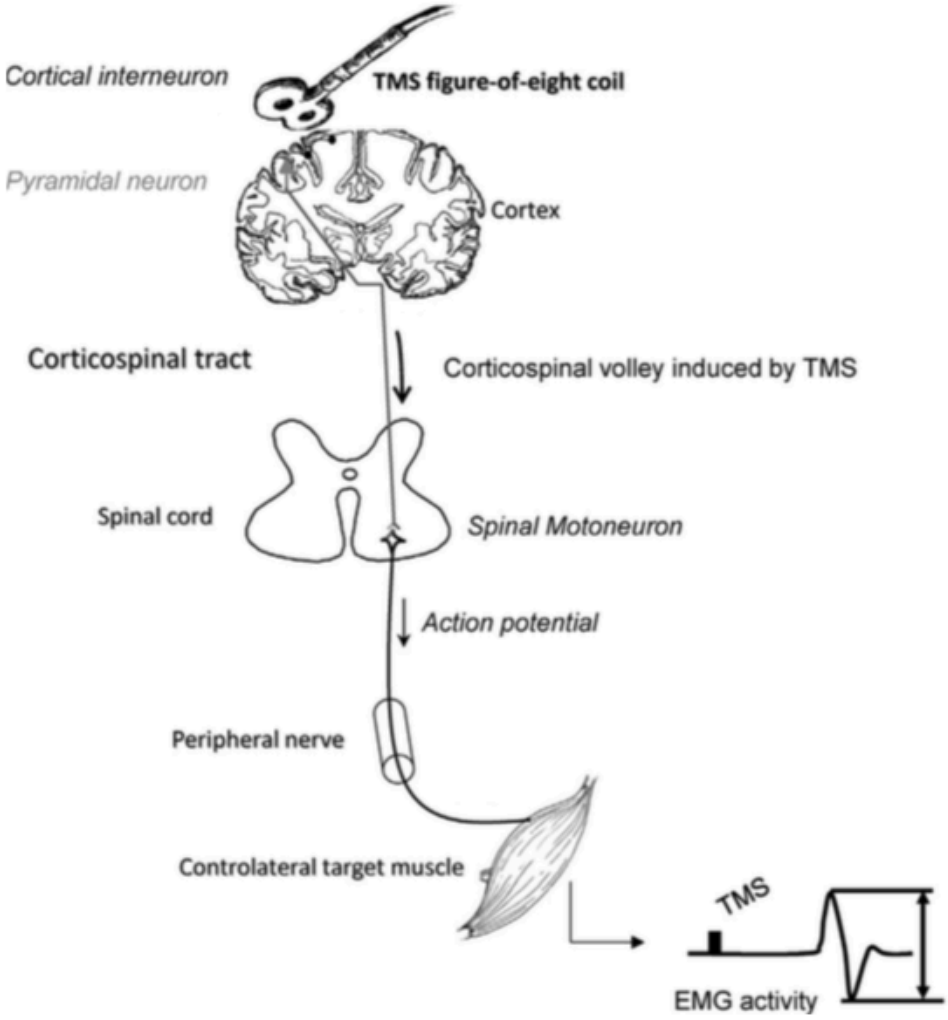

Figure 1.3: A simplictic schematic of RMT – The diagram portrays the elments & the pathway inolved in eliciting MEPs in the RMT measurement. Adapted from Klomjai et. al.

The pharmacological studies have illustrated the involvement of neurotransmitters & neuromodulators in the RMT assesment. Across multiple studies it has now been exhibitied that RMT is not altered by the action of NMDA-antagonists (e.g. - memantine), GABA-ergic agents (e.g. - lorazepam), AchEIs (e.g. - donepezil). Also, dopaminergic & serotoninergic agents are also ineffectve in altering RMT. On the other hand, Na^+ and few other cation channel blockers (e.g. - phenytoin) increase the RMT implying that ion conductance of neuronal membrane mediates RMT (Ziemann, 2004). It has been shown that ketamine lowers the RMT. It has been reasoned out that ketamine by acting on NMDARs causes AMPARs to increase their activity, thereby lowering RMT (Lazzaro et al., 2003).

Hence, the most likely neuronal substrates involved in the case of RMT are –

- the axonal membrane excitability of the cortico-cortical neurons (layers 2-3) & that of pyramidal (cortico-spinal) neurons (layer 5)
- AMPAR-mediated synaptic activity.

The hyperexcitability notion in the case of AD is expressed as decrease in the RMT with enhancing disease stage. Keeping in view the above-mentioned neuronal substrates, the lowering of RMT in AD cannot be explained by AMPAR-mediated synaptic activity as AD is characterized by AMPAR downscaling & dysregulation (Chang et al., 2006). This means that reduction in the AMPAR activity in AD should lead to increase in the RMT that opposes the empirical data. Hence, the most plausible candidate for altered RMT in AD is the increase in axonal membrane excitability of the neurons. The recent literature in AD, provides several contributors of AD pathology that can explain the increase in membrane excitability. The factors that can *modulate* an increased excitability include –

Changes in the membrane structure (& thereby function) caused by amyloid derived diffusable ligands (ADDLs) & other amyoid oligomers (Ferreira and Klein, 2011).

Effect of the altered conducted of the voltage-gated sodium channels (VGSCs) of the microglial cells on the neighbouring neurons, as a consequence of neuroinflammation (Kim et al., 2007).

Abrupt chnages in the osmolarity due to deposits such a plaques & tangles.

Malfunctioning of Na-K pumps resulting from multiple pathologies (Vitvitsky et al., 2012).

Imbalance in calcium homeostasis altering membrane polarity (Bezprozvanny and Mattson, 2008).

More unique is the role of factors that can create increased membrane excitability –

Amyloid – beta oligomers can form non-spefic, heterogeneous cationic ion channels in the membranes of neurons in cases where plaque formation does take place (Lal et al., 2007) (Ferreira and Klein, 2011).

Tau proteins can form pores in the neuronal membrane that allow passage of small ions (Patel et al., 2015) (Maccioni et al., 2010).

Some or all of these factors can explain the recorded lowering of RMT in AD (Figure 1.4).

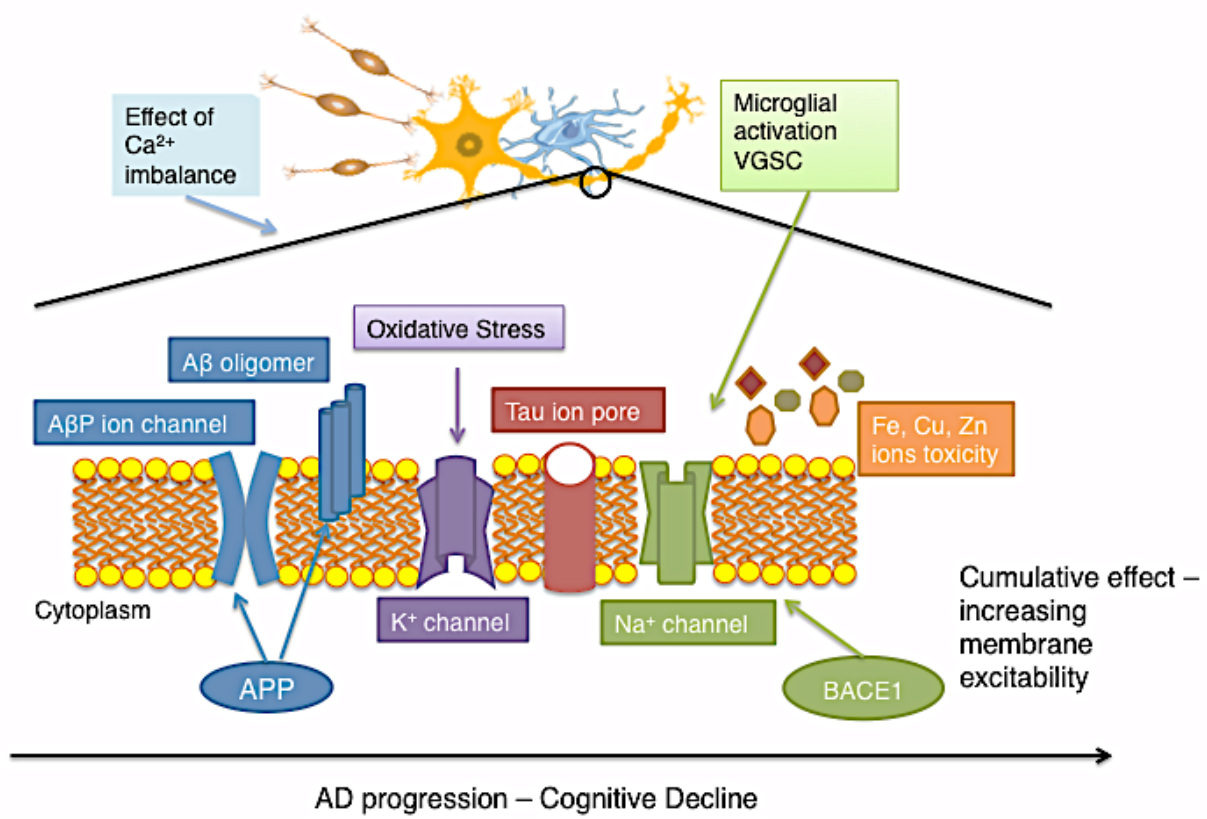

Figure 1.4: Effects of AD pathology on neuronal membrane – A hypothesized cascade of pathological developments in AD that can have cumulative effect resulting in increasing membrane excitability with increasing severity of disease.

The differential vulnerability of different neuronal topographies to amyloid or Tau driven neuropathology, mark a spatial sequence of AD progression in the brain. AD associated pathologies are developed early-on in the hippocampal & medial temporal lobe regions and eventually reach neocrtical areas (Braak et al., 2006). Motor cortex is considered to be affected in later stages. Hence, neuronal membrane excitability measured as RMT in the motor cortex might correlated with global cognitive decline, which in turn is a later consequence in AD evolution.

This thesis spans to ask two broad questions with relevant sub-questions –

- Is RMT correlated/associated with (global) cognitive decline in AD?
    - Is RMT an independent marker/determinant of cognitive decline or a mere proxy for some other marker (cortical thinning or brain shrinkage)?

- Does this correlation differ across cognitive domains (e.g., memory or executive function)?
- Is correlation viable across different RMT protocols, cognitive tests & subject populations ?

- Can RMT act as a discriminator between AD & healthy aging?
  - Is this discrimination at the group level/ individual level?
  - What factors determine the strength of discrimination?

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2. Materials & Methods

For better accessibility, this section is divided into the two broader questions that this thesis attempts to answer.

2.1 *Association between resting motor threshold (RMT) and cognitive decline in AD*

To understand the relationship between motor cortical excitability as measured by RMT and cognitive decline assessed by neuropsychological testing, we conducted a three-tier analysis of retrospective data.

a. Test Cohort - This cohort consisted of a group of 21 participants diagnosed with a probable AD of mild to moderate stage and a comparison/control group of 27 cognitively normal (CN) participants. The cohort was purposed to test our hypothesis for the relationship between RMT and the global cognitive decline in AD in research detail.

Data acquirement

Human Information

The participants were involved in studies conducted at BA-CNBS between 2012 & 2015. Details regarding recruitment procedure & integrity have earlier been published in (Fried et al., 2017). All participants underwent a comprehensive neuropsychological, structured neurological, & medical history evaluation. Those negative for motor and gait impairment, presence of comorbidities & use of CNS (central nervous system) affecting drugs were included in the study. Further, participants in the AD group were tested for a CDR (Clinical Dementia Rating) = 1.0. MRI scans were used to exclude AD participants portraying any abnormality other

than atrophy. Only one AD participant was under the course of a diabetic drug. In the CN group, cognitive intactness was determined by Mini-Mental State Examination (MMSE) scores ranging between 27 & 30. For a subset of the cohort (n_{AD} = 12, n_{CN} = 21), genotypic testing was conducted to assess the BDNF (Brain Derived Neurotrophic Factor) & ApoE (Apolipoprotein E) polymorphisms. Cohort information for measurements used in this study is summarized in Table 3.1.

Imaging

A T1-weighted anatomical MRI scans were obtained in all participants on a 3T scanner (GE Healthcare, Ltd., UK) using a 3D spoiled gradient echo sequence: 162 axial-oriented slices for whole-brain coverage; 240-mm isotropic field-of-view; 0.937-mm x 0.937-mm x 1-mm native resolution; flip angle = 15°; TE/TR ≥ 2.9/6.9 ms; duration ≥ 432 s. T1-weighted anatomical MRIs were analyzed with Freesurfer 6.0 (freely available online at http://surfer.nmr.mgh.harvard.edu/ for download & documentation). To ensure overall accuracy of segmentations and parcellations, all reconstructions were subjected to a rigorous data quality control process: a trained rater reviewed and manually corrected reconstructions when necessary, which were reviewed by another evaluator.

Measurement of cortical thickness (CT in mm) for the left hemisphere (LH) was performed in Freesurfer 6.0. Handknob (Hk) ROIs were extracted from existing template and mapped on the individual T1 scans in the surface space to measure the CT for a particular region of the LH motor cortex.

Neuropsychological Testing

Alzheimer's Disease Assesment Scale-Cognitive Subscale (ADAS-Cog) was the primary neuropsychological test used as a measure of the global cognitive decline in AD for the reasons explained below. This is a comprehensive eleven item test evaluating multiple domains of cognition including attention, memory, comprehension, orientation, language, etc. The total score range for ADAS-Cog scale is 0-70 where a higher score corresponds to greater cognitive dysfunction. The specificity of ADAS-Cog for dementia due to AD compared to other forms of

dementia and its sensitivity towards capturing the multiple dimensions of cognitive decline compared to other neuropsychological tests is significantly greater.

Additionally, NACC-UDS Battery (National Alzheimer's Coordinating Center – Uniform Data Set) was administered to all the participants. The battery package comprises several separate tests – Mini-Mental State Examination (MMSE) being a test for measurement of global cognition (score range – 0-30; lower score corresponds to greater cognitive dysfunction), Rey Auditory Verbal Learning Test (RAVLT) for assessing learning an memory and other tests like Boston Naming Test, verbal fluency tests, trail making tests, etc. for assessing executive function decline. The participants were also assessed for depression associated with AD using GDS (Geriatric Depression Scale; score: 0 – 5). W-TAR (Weschler-Test of Adult Reading) was administered to evaluate the pre-morbid IQ of the participants.
No. of missing data cases for the participants across groups is reported in Table 3.1.

TMS & associated measurements

TMS was conducted using Nexstim Monophasic System (Nexstim Inc, Finland) for measuring both monophasic RMT (m-RMT) and biphasic RMT (b-RMT). Both measurements were performed using a figure-of-eight focal coil placed tangentially over the scalp surface. Navigated Brain Stimulation (NBS) System (Nexstim Inc., Finland) was used for finding the stimulation hotspot (~ cortical area representation of the target muscle to generate the highest MEP for given stimulation intensity) in the motor cortex of the left hemisphere (LH) using the high-resolution T1-MRI images for individual participants. MEPs (as measured using EMG) were recorded for the relaxed FDI (first dorsal interosseus) muscle (right hand) as the target, with APB (abductor pollicis brevis) or ADM (abductor digiti minimi) muscles as references. RMTs (expressed as %MSO) were recorded according to the current IFCN standards by the relative frequency method as defined above in section 1.2. The inter-pulse interval was randomized between 5000-6000 ms to avoid repetitive stimulation.

The electric field localization values (EF_{dist}) and the electric field maximum intensity (EF_{max}) calculated from the output of the NBS system for every trial that contributed to the RMT. EF_{dist} represents the Euclidean distance between the localization point

of the center of the coil (placed over the scalp) and the localization point in the cortex where electric field intensity (EF_{max}) delivered was maximum for stimulation (Equation 1). Coil to cortical stimulation distance (CCD in mm) was calculated as the average of EF_{dist} values across RMT trials (Equation 2). A similarly computed average of EF_{max} was defined as motor threshold expressed in E-field values, EF_{MT} (in V/m) (Equation 2).

$$EF_{dist} = \sqrt{(E_x^c - E_x^s)^2 + (E_y^c - E_y^s)^2 + (E_z^c - E_z^s)^2} \quad \text{(Equation 1)}$$

(x,y,z) = co-ordinates
c = coil localization
s = cortical stimulation localization

$$CCD = \sum_{i=1}^{n} \frac{EF_{dist}^{i}}{n}$$

$$EF_{MT} = \frac{\sum_{i=1}^{n} EF_{max}}{n} \quad \text{(Equation 2)}$$

n = number of trials that correspond to % MSO value used for RMT

The scalp to cortex distance (SCD) was measured manually by an independent rater for all participants. The coordinates of the stimulation localization mapped on the anatomical scans, as obtained from the system were transported to Brainsight (https://www.rogue-research.com/) for visualization. In the Brainsight space, the coordinates for the point on the scalp directly above the stimulation localization point on the cortical surface was marked. SCD (in mm) was measured as the difference between the coordinates of the two points representing the shortest – perpendicular distance.

Data analysis

Parametric tests, t-test for continuous variables & chi-squared test for categorical variables, were employed to compare the AD & the CN groups for relevant measures.

Primary Hypothesis: Is ADAS-Cog correlated to RMT in AD?

Ordinary Least Squares (OLS) simple linear regression was performed to check the association between ADAS-Cog and m-RMT across both AD & CN group (Equation 3). The standardization of ADAS-Cog scores was according to (Equation 4). Further, separate multiple linear regression models (Equation 5) were used to assess the individual influence of each covariate/factor on this (ADAS-Cog – m-RMT) association. Age, gender, hand-dominancy, BDNF – Val status, ApoE – ε4 status, SCD, CCD & CT-LH. The choice for the covariates follows from the discussion in section 1.2. In a case where the effect of a covariate was observed to be beyond tolerance, i.e., % $\Delta\beta_{RMT} > 10$ (Equation 6), the covariate was assessed for mediation using bootstrapping or for confounding using OLS regression with RMT as the dependent variable & covariate as the independent variable.

Basic model → cognitive score (z-value) = $\beta_o + \beta_{RMT}.RMT$ (Equation 3)

Updated model → cognitive score (z-value) = $\beta'_o + \beta'_{RMT}.RMT + \beta_{covar}.covariate$ (Equation 4)

Z-value = $\frac{x - \mu_{CN}^{x}}{SD_{CN}^{x}}$ (Equation 5)

$\mu_{CN} =$ variable mean for CN group of test cohort

$SD_{CN} =$ standard deviation for the variables in the CN group

%Δ $\beta_{RMT} = (\frac{\beta_{RMT} - \beta'_{RMT}}{\beta_{RMT}}) \times 100$ (Equation 6)

Exploratory Analysis: Is RMT correlated with specific domains of cognitive function?

MANOVA was performed to evaluate the relationship between m-RMT and composite memory score (CMS), composite executive function score (CES) & ADAS-Cog. This design allowed to check if the association was comparatively 'stronger' for RMT and memory, global cognition or executive function.

The composite memory score (CMS) was calculated as the average (Equation 7) of the z –values (standardized) for RAVLT Immediate Recall, RAVLT Delayed Recall, RAVLT Recognition, Logical Memory Immediate Recall & Logical Memory Delayed Recall. While the composite executive function score (CES) was calculated as the

average of the z values for Trail Making Time A, Trail Making Errors A, Digit Span Forward Length, Digit Span Backward Length, Boston Naming Test, Verbal Fluency (Animals) & Digit Symbol Substitution Test (DSST).

$$\textit{Composite score} = \sum_{i=1}^{n} \frac{z_i}{n} \qquad \text{(Equation 7)}$$

z_i = z value for component (cognitive test) score

n = No. of individual cognitive test used as components for the composite

Both analyses were repeated for biphasic RMT to evaluate the sustainability of results across TMS pulse shapes.

b. Validation Cohort - This cohort consisted of 128 participants, diagnosed with a probable AD of mild to moderate stage, involved in a multi-center TMS trial sponsored by Neuronix Ltd. between October 2013 & March 2016. The details of the trial can be found at – ClinicalTrials.gov (https://www.clinicaltrials.gov/ct2/show/NCT01825330?term=NCT+01825330&rank=1). The purpose of the inclusion of this cohort was to check the validity of the findings from the test cohort in a more general and clinically relevant population setting.

Data acquirement

TMS measurements across all centers were performed using Magstim Rapid 2 system (Magstim, Inc - US) with an air-cooled figure-of-eight coil. Motor cortex of the left hemisphere was stimulated for the determination of RMT using visual assessment of FDI muscle twitch. No EMG was used for MEP amplitude evaluation. A neuronavigation system did not guide motor hotspot search.

BA-CNBS was one of the partnering centers for the trial, and hence we had limited access to the data of the entire trial. For this cohort, the available data includes - biphasic RMTs (75.52 ± 12.19 %MSO), demographic characteristics such as – age (76.83 ± 6.88 years), gender (46.1% females), AD medication status (20.3% on AD medication) & neuropsychological assessments - ADAS-Cog (23.71 ± 5.29) &

MMSE (21.57 ± 2.46). AD medication included AchEIs (e.g., donepezil, rivastigmine or galantamine), NMDA antagonist (memantine) or a combination of both. There was no overlap between the test and the validation cohorts considering the participants.

Data Analysis

Hypothesis: Is ADAS-Cog correlated with RMT in AD? (same as for the test cohort)

The analysis was similar to the one performed for the test cohort. The relationship between ADAS-Cog & b-RMT was assessed using OLS linear regression and subsequent (single covariate) multiple regression models using age, gender and medication status as separate covariates. The ADAS-Cog scores were standardized as described above.

For both cohorts, all p values in case of group comparisons and multiple regression were corrected for multiple comparisons using Holm-Bonferroni correction. For all analysis inferences, the level of significance was $\alpha = 0.05$. Standardization of the cognitive scores (e.g., ADAS-Cog, CMS, CES) was carried out to facilitate interpretation of results for both the cohorts.

c. Evaluation Cohort – This cohort is a group of previously published studies in addition to the data presented here, that have assessed the correlative relation between cognitive decline and RMT. Quantitative synthesis (meta-analysis & meta-regression) was conducted for these studies to evaluate the pooled coefficient of correlation and account for probable heterogeneity across studies.

Data Acquirement

Data acquirement was conducted according to the PRISMA (Preferred Reporting Items for Systematic Review & Meta-Analysis - http://www.prisma-statement.org/) guidelines. The research design – selection of sources for data identification, distribution of screening activities, inclusion criteria for study selection, questions to be analyzed, etc. were set *a priori* adhering to the PRISMA guidelines. Two investigators worked together for acquiring the data. Pubmed & Web of Science were

chosen as the primary sources for identification of studies. The key search term used for both databases was – “magnetic stimulation AND Alzheimer.” No other available filters were used. This search strategy was implemented to maintain consistency with previously published systematic review involving the same topic (Freitas et al., 2011). The database search was conducted been January & February 2018. Each investigator was assigned a database for screening. Studies were screened by the relevancy of their titles & abstracts. Studies identified from both the databases were pooled together in a citation manager & similarity of study titles was used to remove the duplicates. After removing duplicates across databases, evaluation of the merged list was conducted by one investigator for reaffirming our earlier screening. In selection procedure, studies were screened for their content in entirety to assess their eligibility for inclusion. The studies satisfying all the inclusion criteria were used for the quantitative synthesis. Additionally, references of selected studies were screened as additional data sources to acquire any studies not captured in the database search. An overview of the workflow is given in Figure 2.1.

Question for meta-analysis - What is the effect size of the correlation between RMT & cognitive decline in AD? How is this correlation moderated by demographic factors and stimulation factors (or put equivalently, do these factors account for the heterogeneity among studies)?

Inclusion Criteria – The study should comprise of -

1. Subjects/Participants with a probable or clinical diagnosis of Alzheimer’s Disease (AD)
2. Resting motor threshold measurements performed (irrespective of stimulation parameters) for AD participants
3. Neuropsychology test– MMSE or ADAS-Cog, conducted for these participants
4. Either Pearson correlation values (r) for the relationship between RMT & given test score

   OR

   Raw data for all AD participants (in the form of a list or in a figure/plot from where it can be extracted) for RMT & cognitive score values.

In addition to these, in case of studies focussing on follow-up measurements or pre-post tests for drugs, we decided to include data for only the baseline measurements (1st visit or pre-test) to maintain consistency for cross-sectional nature of the data in the analysis. Only one study from the selection had raw data for ADAS-Cog and corresponding RMT values. No other studies reported r values for ADAS-Cog and RMT relationship. Theoretically, a meta-analysis requires at least three studies. Hence, we could not conduct a quantitative analysis with ADAS-Cog as a cognitive decline measure. Seven studies were selected for the meta-analysis of correlation between MMSE score & RMT. MMSE – RMT data from the test & the validation cohorts were added to these 7, for the quantitative analysis.

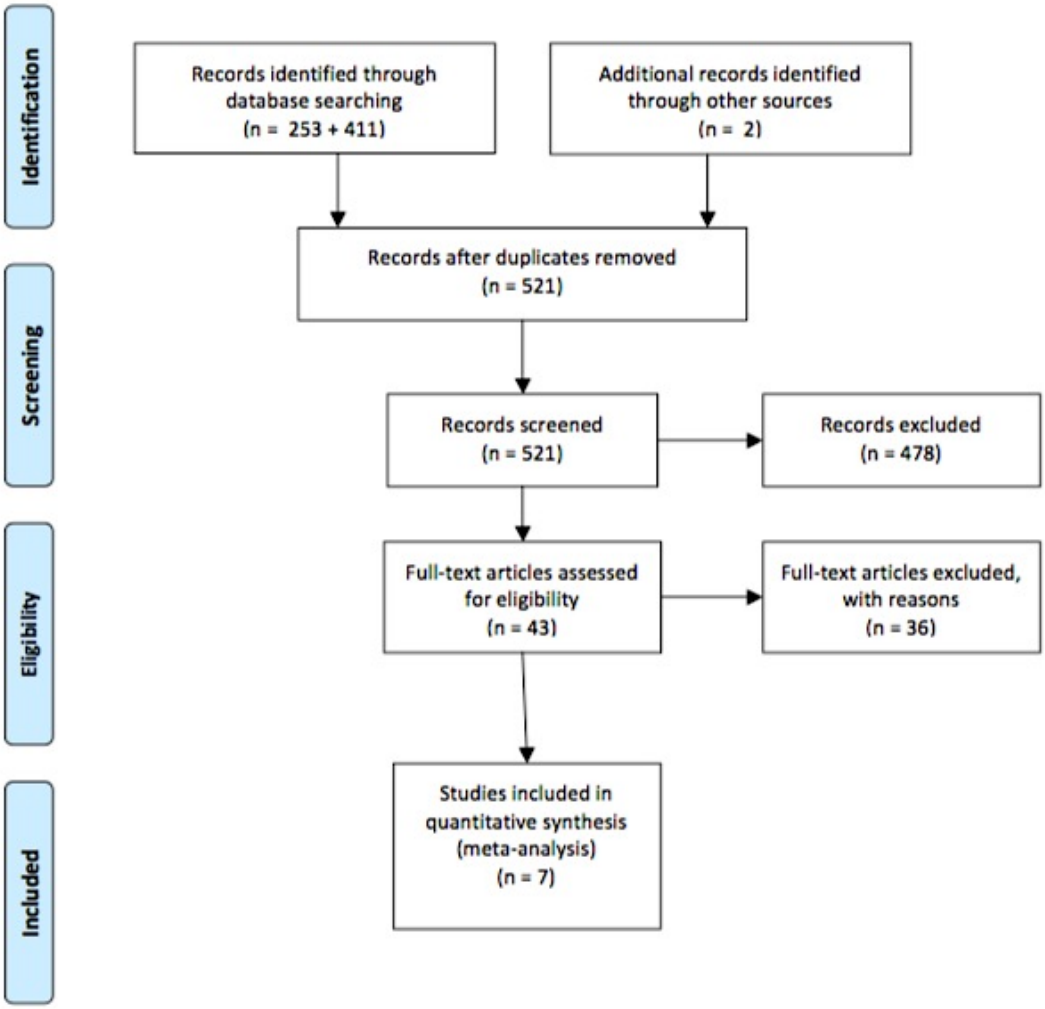

Figure 2.1: RMT- MMSE Correlation PRISMA flow diagram: Flowchart of data selection procedure for the quantitative synthesis of correlation between resting motor threshold (RMT) and cognitive decline (as measured by MMSE) in Alzheimer's Disease.

Data Analysis

Pearson (product-moment) correlation values (r) were calculated for the studies from which raw data was extracted. For other studies, the reported values were used. The individual r values were standardized by Bare-Bones method with modifications suggested by Hunter & Schmidt (HS) for meta-analysis of correlations. In this method the individual r values are standardized using the sample sizes, to prevent the influence of sample size differences across studies. This standardization was especially useful here, given the disparity among selected study sample sizes. Further, HS method provides correction for artifacts (measurement errors) by normalizing the estimated sampling variances by reliability measures known for the variables used for correlation. The reliability for both RMT & MMSE measurements has been earlier shown to be high (~1). Hence, artifact correction was not considered necessary. To get an estimate of the pooled correlation (*r'*) value, a fixed-effects model was fitted to the standardized r values. This model provides narrow confidence intervals for the estimate as the Q-statistic used by it for the assessment of heterogeneity in the total effect size assumes only chance differences between studies. Next, a random effects model was fitted using sampling variances of the studies as the weights, which gives a pooled correlation with a wider confidence interval, since this model takes into account the inter-study variability. HS estimator was used for assessment of total heterogeneity (τ^2). I^2 , defined as % total heterogeneity / total variability & levels of heterogeneity are categorized based on the I^2 value ranges – 0 – 25% : Low heterogeneity, 25 – 50% : Moderate heterogeneity, 50 – 75% : Substantial heterogeneity, 75 – 100% : High heterogeneity. H^2, defined as total variability/sampling variability was also reported. Further, mixed-effects models (meta-regression) (sampling variances used as weights) were used to check if the heterogeneity in the pooled correlation *r'* as obtained through the random effects model can be accounted for by demographic and/or stimulation variables. For meta-regression, three sets of moderators were considered –

a. Set of demographic moderators – This included age (mean values for each study) & gender (expressed as % females in each study). While years of education was also included in the *a priori* list of moderators for this analysis, this information was not available for more than half the studies selected for this analysis.

b. Set of stimulation-dependent moderators – This included coil shape (circular v/s figure-of-eight), pulse shape (monophasic v/s biphasic), RMT definition (IFCN: ≥ 50 μV in at least 5/10 trials v/s other). The protocol for conducting RMT has changed over the years, as our knowledge about TMS effects and safety grew. Hence, there was variability across the selected studies either for the amplitude (e.g.,> 20 μV instead of ≥ 50 μV) or the number of trials used (e.g., 3/6 instead of 5/10) or both. This necessarily would increase the variability of *what* was measured as RMT across studies. The *a priori* list also included neuronavigation and EMG as moderators. However, EMG was included in all except one study (data from our validation cohort). Neuronavigation was not used in any studies, except in the case of data from our test cohort.

c. Set of all moderators – This included all the moderators used above in the demographic and the stimulation-dependent sets. The purpose of the model was to know if all the factors that might account for heterogeneity among studies, when taken together made a better prediction.

The τ^2, H^2, I^2 reported for the models in the case of meta-regression stand for residual heterogeneity instead of total heterogeneity. The R^2 value of the model gives the percentage of the heterogeneity accounted for/predicted by the moderators.

Further, credibility/prediction intervals were calculated for the pooled correlation coefficient estimated by the random-effects model. The entire analysis was reconducted after removing the data from our test and validation cohort to check the consistency of results with only previously published studies as data contributors for quantitative analysis.

2.2 Comparison of resting motor threshold (RMT) between healthy and dementia aging

The strategy for data acquirement and analysis for this quantitative analysis was similar to the quantitative synthesis described above. With changes in the inclusion

criteria, effect size calculation, selection of moderators, necessary for the answering the presented problem.

Data acquirement was conducted according to the PRISMA (Preferred Reporting Items for Systematic Review & Meta-Analysis - http://www.prisma-statement.org/) guidelines. The research design – selection of sources for data identification, distribution of screening activities, inclusion criteria for study selection, questions to be analyzed, etc. were set *a priori* adhering to the PRISMA guidelines. Two investigators worked together for acquiring the data. Pubmed & Web of Science were chosen as the primary sources for identification of studies. The key search term used for both databases was – "magnetic stimulation AND Alzheimer." No other available filters were used. This search strategy was implemented to maintain consistency with previously published systematic review involving the same topic (Freitas et al., 2011). The database search was conducted been January & February 2018. Each investigator was assigned a database for screening. Studies were screened by the relevancy of their titles & abstracts. Studies identified from both the databases were pooled together in a citation manager & similarity of study titles was used to remove the duplicates. After removing duplicates across databases, evaluation of the merged list was conducted by one investigator for reaffirming our earlier screening. In selection procedure, studies were screened for their content in entirety to assess their eligibility for inclusion. The studies satisfying all the inclusion criteria were used for the quantitative synthesis. Additionally, references of selected studies were screened as additional data sources to acquire any studies not captured in the database search. An overview of the workflow is given in Figure 2.2.

Question for meta-analysis - What is the effect size of the resting motor threshold difference between AD & CN groups? How is this difference moderated by demographic factors and stimulation factors (or put equivalently, do these factors account for the heterogeneity among studies)?

Inclusion Criteria – The study should comprise of -

1. Subjects/Participants with a probable or clinical diagnosis of Alzheimer's Disease (AD) accompanied by a group of cognitively normal (CN) old controls

2. Resting motor threshold measurements performed (irrespective of stimulation parameters) for AD & CN participants, where RMT was measured by stimulating the left hemisphere (LH)
3. The mean and standard deviation (SD) values for AD & CN groups

OR

Any other data (raw data, report of SE or CI instead of SD, etc.) for all AD & CN participants for RMT values which can be used to calculate the mean & SD values for the groups separately.

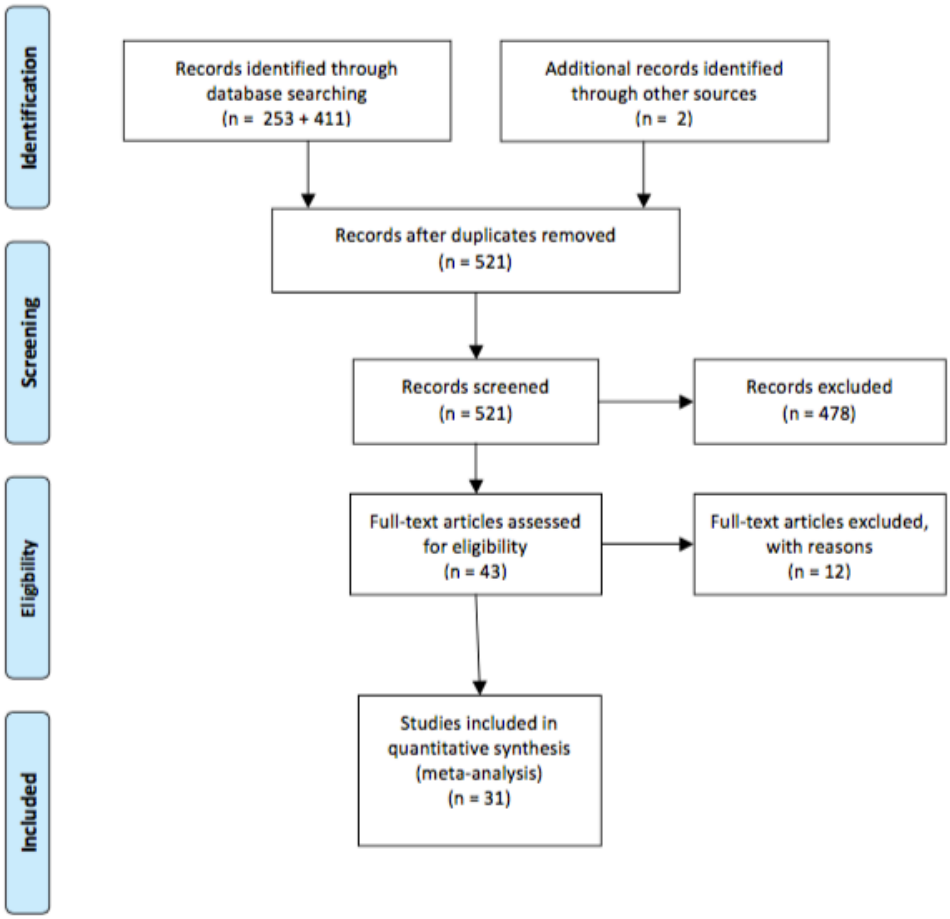

Figure 2.2: RMT comparison PRISMA flow diagram: Flowchart of data selection procedure for the quantitative synthesis of standardized mean difference in resting motor threshold (RMT) between Alzheimer's Disease (AD) & cognitively normal (CN) groups.

In addition to these, in case of studies involving follow-up measurements or pre-post tests for drugs, we decided to include data for only the baseline measurements (1st visit or pre-test) to maintain consistency for cross-sectional nature of the data in the analysis. The studies which reported only mean values and for which SD of the RMT

could not be calculated were discarded as well. Studies that could be identified as involving same/overlapping participant populations were clubbed together as one. Two studies were discarded for likely overlap with our test cohort data. To the 31 studies finally selected for the quantitative analysis, data from our test cohort was added, making it 32 studies.

Data Analysis

Standardized mean differences were calculated for the studies using the SDs & sample sizes of the AD & CN groups. The estimate of the 'total' standardized mean difference (SMD) as calculated here is commonly referred to as Hedge's G. Initially, a fixed-effects model was fitted to the SMDs. This model provides narrow confidence intervals for the estimate as the Q-statistic used by it for assessment of heterogeneity in the total effect size assumes only chance differences between studies. Next, a random effects model was fitted, which gives a Hedge's G with a wider confidence interval, since this model takes into account the inter-study variability. Hedges's estimator was used for assessment of total heterogeneity (τ^2). I^2 , defined as % total heterogeneity / total variability & levels of heterogeneity are categorized based on the I^2 value ranges – 0 – 25% : Low heterogeneity, 25 – 50% : Moderate heterogeneity, 50 – 75% : Substantial heterogeneity, 75 – 100% : High heterogeneity. H^2, defined as total variability/sampling variability was also reported. Further, mixed-effects models (meta-regression) were used to check if the heterogeneity in the Hedge's G as obtained through the random effects model can be accounted for by demographic variables. For meta-regression, following models were considered.

a. MMSE Score: To check if the cognitive decline in AD as measured by MMSE scores could explain the heterogeneity in the SMDs for RMT measurements across studies. MMSE scores are reflective of the disease stage, which might influence the hyperexcitability as measured by RMT for the AD groups across studies, in turn affecting the SMD values.

b. Years of Education: Since years of education is considered a marker of cognitive reserve, apart from being a measure of pre-morbid IQ. The cognitive reserve is thought to have a neuroprotective effect in AD, and hence it was considered relevant to check if it explains heterogeneity in RMT SMDs.

c. Age

For the above moderators, mean values of these measures for the AD groups across studies were used.

d. Gender: This was expressed as a percentage of females in the AD groups across studies.

e. Total: This model combined all the moderators to assess if these measures taken together make a better prediction of the heterogeneity.

Compound stimulation parameter scores ($W_{stimulation}$) were used as weights for the random & mixed-effects models. The scores were calculated as described below –

$$W_{stimulation} = score_{\ neuronavigation} + score_{\ RMT\ definition} + score_{\ coil\ shape}$$

$Score_{neuronavigation}$	Neuronavigation present → 1 Neuronavigation absent → 0
$Score_{RMT\ definition}$	According to recent ICFN criteria → 1 Others → 0
$Score_{coil\ shape}$	Figure-of-eight coil → 1 Circular coil → 0

A higher score reflects better stimulation standard. Neuronavigation has been shown to increase the precision of searching & targetting the motor hotspot. The figure-of-eight coils are known to result in more focal stimulation compared to the circular coils. The current operational definition of RMT takes the accuracy of measurement and safety of stimulation into account. Hence, these compound scores believed to weigh studies according to the *quality of stimulation*.

The τ^2, H^2, I^2 reported for the models in the case of meta-regression stand for residual heterogeneity instead of total heterogeneity. The R^2 value of the model gives the percentage of the heterogeneity accounted for/predicted by the moderators. Further, credibility/prediction intervals were calculated for the Hedge's G (SMD) estimated by the random-effects model.

Statistical analysis was performed using R (version – 3.4.3) (https://www.r-project.org/) packages in R-studio (version – 1.1.383). All packages are open-sourced & have been validated across publications & users.The cortical thickness measurements were acquired from Dr. Stephanie Buss.Dr. Peter Fried conducted the scalp-to-cortex measurements. Katherine McDonald was the second reviewer for identification and screening of studies in case of both the quantitative synesthetes. Arianna Menardi contributed to data selection and qualitative overview.

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3. Results

3.1 *Association between resting motor threshold (RMT) and cognitive decline in AD*

a. Test Cohort

After adjusting for multiple comparisons, the AD & the CN groups differed significantly ($p < 0.0001$ for each test) in their neuropsychological assessments - ADAS-Cog, MMSE & CMS. The cortical thickness of the left hemisphere (CT-LH) was significantly lower in the AD group ($df = 42$, $t = 4.72$, $p = 0.0005$) depicting atrophy in the cortical grey matter. Apart from these, ApoE ε4 status differed significantly ($\chi^2 = 13.45$, $p = 0.005$) between the two groups. Comparison of all other measures including RMT resulted in non-significant differences between the two groups. The results are summarised in Table 3.1.

	Alzheimer's Disease Participants (AD)	Cognitively Normal Participants (CN)	Group Comparison		
	Measures - Mean (SD) or no. (%)		df	t/ χ^2 Value	Adjusted p
Demographic Measures					
Total Number	21	27			
Females (%)	13 (61.9)	13 (48.1)	-	0.43	> 0.05
Right-hand-dominanats (%) (n =47)	17 (85)	27 (100)	-	2.18	> 0.05
Age	69.67 (7.54)	62.37 (9.09)	46	-3.04	> 0.05
Years of Education	16.62 (3.81)	15.85 (2.38)	32	-0.81	> 0.05

W-TAR (Weschler-Test of Adult Reading)	108.24 (15.24)	113.81 (10.12)	33	1.45	> 0.05
GDS (Geriatric Depression Scale)	2.43 (2.34)	0.52 (0.89)	25	-3.55	0.032
ADL (Activities of Daily Living)	69.62 (6.79)	75.89 (2.85)	25	3.97	0.017
Genotypic Measures					
BDNF - Met ≥ 1 (%) (n_{AD} = 12, n_{CN} = 21)	5 (41.6)	2 (9.5)	-	2.99	> 0.05
ApoE- ε4 ≥ 1 (%) (n_{AD} = 12, n_{CN} = 21)	11 (91.7)	4 (19.1)	-	13.45	0.005
Neuropsychological testing Measures					
ADAS-Cog (Alzheimer's Disease Assessment Scale - Cognitive Subscale) (n_{AD} = 20)	22.60 (10.55)	3.95 (1.79)	20	-7.83	3.50E-06
MMSE (Mini-Mental State Examination)	21.76 (2.49)	29.48 (0.75)	23	13.74	3.43E-11
Composite Memory (CMS) Scores (z - values)	-3.56 (1.48)	0 (0.56)	25	10.45	3.36E-09
Composite Executive Function (CES) Score (z - values) (n_{AD} = 18)	-0.70 (1.09)	0 (0.31)	20	2.73	0.182
Neuroanatomical Measures					
Scalp to Cortex Distance (SCD) (mm)	16.18 (2.3)	16.11 (3.0)	46	-0.08	> 0.05
Cortical Thickness (CT) - Left Hemisphere (LH) (mm)	2.18 (0.08)	2.3 (0.08)	42	4.72	4.98E-04
Cortical Thickness (CT) - Motor cortex Handknob region (Hk) (mm)	2.29 (0.16)	2.39 (0.13)	38	2.39	0.286

TMS Measures					
Resting Motor Threshold (RMT) - Monophasic (% MSO)	62.48 (13.91)	64.85 (14.63)	44	0.57	> 0.05
Resting Motor Threshold (RMT) - Biphasic (% MSO)	43.43 (10.51)	44.85 (9.55)	41	0.48	> 0.05
Coil to Cortical-stimulation Distance (CCD) - Monophasic (mm)	26.44 (2.43)	27.6 (3.1)	46	1.46	> 0.05
Coil to Cortical-stimulation Distance (CCD) - Biphasic (mm)	25.41 (3.36)	26.52 (3.26)	42	1.15	> 0.05
EF_{MT} Monophasic (V/m)	74.84 (20.96)	75.94 (24.07)	45	0.17	> 0.05
EF_{MT} Biphasic (V/m)	65.85 (22.46)	66.46 (19.39)	40	0.1	> 0.05

Table 3.1: Group Characteristics of Test Cohort – All the measures are presented as mean (SD) or absolute no. (%). The p values given here are corrected for multiple comparisons. In case of missing data cases, the no. of observations used for comparison is specified. Green markers statistically significant p values, while orange marks trending significance.

Resting motor threshold has a significant independent relationship with cognitive decline in AD

Simple linear regression revealed a significant relationship between ADAS-Cog and m-RMT in the AD group (df = 1,18, β_{RMT} = - 0.26, Adj. R^2 = 0.35, p = 0.004). Pearson correlation (r) for this relationship was -0.62 (p = 0.0039) indicating that a lower m-RMT was associated with a higher ADAS-Cog score, which in turn is depictive of greater cognitive decline/dysfunction. This relationship was non-significant for the CN group (df = 1,25, β_{RMT} = - 0.01, Adj. R^2 = - 0.01, p = 0.511) (Figure 3.1). This confirmed our primary hypothesis. In the single covariate multiple regression for the AD group, SCD, CT-LH & EF_{MT} –Mono were the only covariates found to have an above tolerance (SCD: $\%\Delta\beta_{RMT}$ = - 15.38%, CT-LH: $\%\Delta\beta_{RMT}$ = 19.23%, EF_{MT}: $\%\Delta\beta_{RMT}$ = 34.62%) influence on the mRMT-ADAS-Cog relationship. However, all the three covariates themselves were non-significant predictors of ADAS-Cog in the presence of m-RMT. CT-LH (Adj. R^2 = 0.18, p = 0.037) & EF_{MT}

(Adj. R^2 = 0.32, p = 0.005) had a significant/trending relationships with ADAS-Cog in the absence of m-RMT, but not SCD. CT-LH was not a significant predictor of m-RMT (Adj. R^2 = 0.01, p =0.3) and hence the most likely inference is that cortical thickness is an independent predictor of cognitive decline, weaker than the m-RMT in this case. EF_{MT} was found to be a strong & significant predictor of m-RMT (Adj. R^2 = 0.75, p < 0.0001). Bootstrap mediation analysis (non-parametric bootstrap confidence intervals with the percentile method) failed to show the indirect effect (mediating effect) of EF_{MT} on the motor threshold – cognition decline relationship. The average causal mediation effect (ACME: b = 0.1, p = 0.21) was non-significant against a highly significant average direct effect (ADE: b = - 0.71, p < 0.0001) Hence, EF_{MT} most likely is a confounding variable which the same phenomenon as m-RMT. This inference is compliant with our knowledge about both EF_{MT} & RMT. The details of multiple regression analysis are summarised in Table 3.2. Hence, this further supports our claim that the relationship between RMT & cognitive decline, although might be influenced by cortical atrophy, is an independent relationship that might represent unique underlying neuropathology in AD.

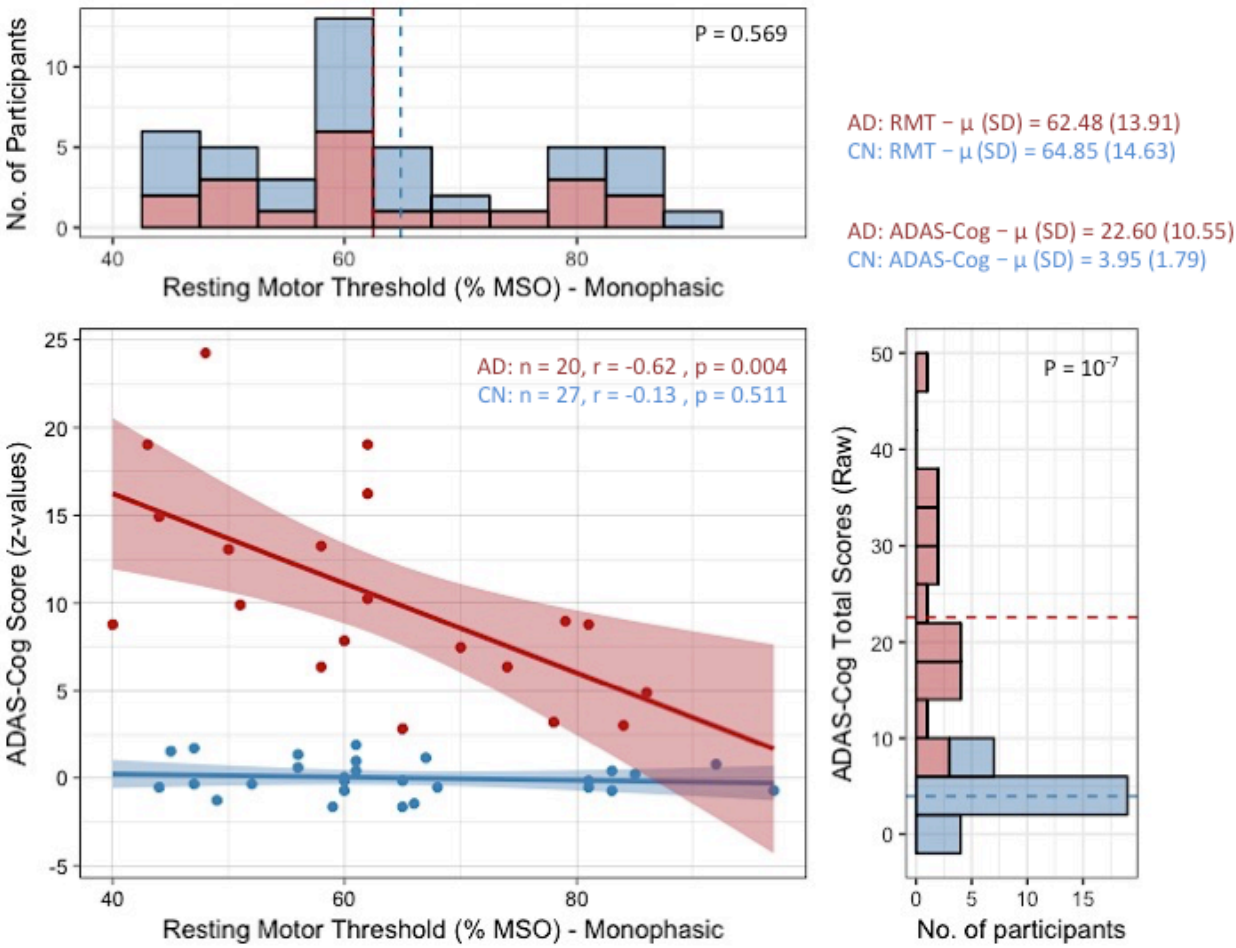

Figure 3.1: Correlation between m-RMT & ADAS-Cog in test cohort – The scatterplot represents the correlation between ADAS-Cog (z- values) and Monophasic RMT as found by simple linear regression (95% CI provided) for AD (red) & CN (blue) groups. The histograms in the side panels provide the distributions of the raw values for both variables with a comparison of how the AD & the CN groups differ for them (uncorrected p -values).

Model Regressors	df	β_{RMT}	% $\Delta\beta_{RMT}$	Adj. R^2_{model}	partial R^2_{covar}	p_{model}	p_{RMT}	p_{covar}
m-RMT	1,18	-0.26	-	0.35	-	0.004	0.004	-
m-RMT & Age	2,17	-0.26	0	0.32	0.02	0.028	0.032	1
m-RMT & Gender	2,17	-0.24	7.69	0.35	0.06	0.028	0.036	1
m-RMT & Hand-dominance	2,16	-0.25	3.85	0.3	0	0.028	0.036	1
m-RMT & ApoE- ε4	2,9	-0.26	0	0.2	0.26	0.076	0.11	1
m-RMT & BDNF- Met	2,9	-0.27	-3.85	0.36	0.4	0.076	0.093	1
m-RMT & CCD - Mono	2,17	-0.25	3.85	0.34	0.04	0.028	0.035	1

m-RMT & SCD	2,17	-0.3	-15.38	0.43	0.18	0.018	0.009	0.612
m-RMT & CT (LH)	2,17	-0.21	19.23	0.39	0.12	0.024	0.06	1
m-RMT & EF_{MT} - Mono	2,17	-0.17	34.62	0.32	0.02	0.028	0.313	1

Table 3.2: Multiple regression for m-RMT – ADAS-Cog relationship in AD –Covariates/factors were used in separate models to check their individual influence on the relationship. The % Δβ > 10 is an indirect measure of greater influence (orange). Partial R^2_{covar} measures the additional covariance explained by the covariate/factor. All p values for covariate models are corrected (green = highly significant p values).

Resting motor threshold might be better correlated with the global cognitive decline

The exploratory analysis to test the relatioship between m-RMT and tests specific for creatin cognitive functions/domains couldn't find a significant difference among ADAS-Cog – m-RMT, CMS – m-RMT & CES – m-RMT relationships (df = 3,14, Pillai's test-stat. = 0.39, p = 0.066). However, this underpowered result is most likely due to a small sample (n_{AD} = 16) used for this analysis. Univariate statistics (Figure 3.2) following MANOVA depict that the individual relationship was most significant for ADAS-Cog (df = 1,16, β_{RMT} = - 0.23, Adj. R^2 = 0.33, p = 0.007), followed by CMS (df = 1,16, β_{RMT} = 0.05, Adj. R^2 = 0.19, p = 0.04), followed by CES (df = 1,16, β_{RMT} = 0.01, Adj. R^2 = - 0.01, p = 0.382). While no concrete inferences can be drawn from this exploratory analysis, it is worth mentioning that the univarite results fall in line with the *membrane excitability rationale* presented above.

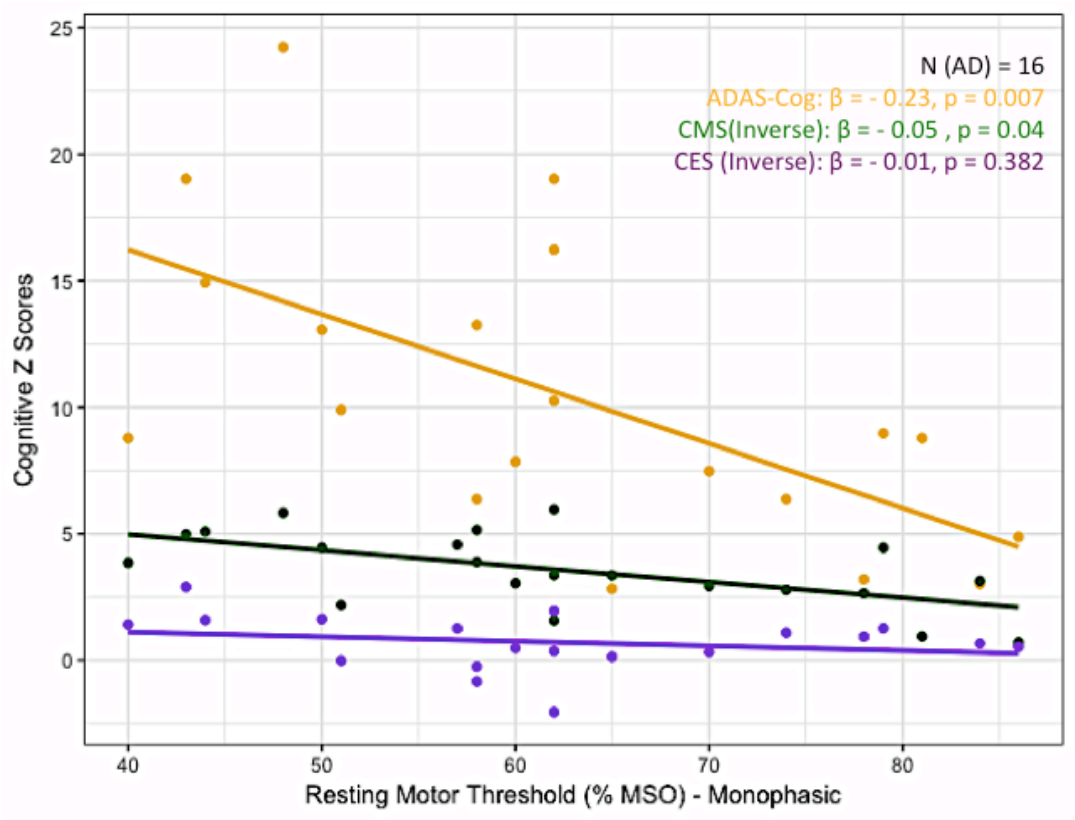

Figure 3.2: m-RMT correlations across different cognitive test measures – Univariate statistics summary from MANOVA gives individual correlations for m-RMT with ADAS-Cog, CMS & CES. For better representation, the CMS & CES scores are inverted. Hence, for all tests, a higher Z score here represents greater dysfunction.

RMT – measuring cortical vs. corticospinal hyperexcitability in AD

While there was no direct way of answering this question in the given cohort, two indirect tests were used –

a. Relationship between EF_{MT} – Mono & ADAS-Cog was evaluated, corrected for SCD (~correction for attenuation of current due to CSF). EF_{MT} showed a significant relationship with ADAS-Cog (df = 2,18, $\beta_{EF\text{-}MT}$ = - 0.18, p = 0.003) while SCD didn't show a significant relationship. The Adj. R^2 for the model (EF_{MT} + SCD) was 0.35 (p = 0.01) & the partial correlation for EF_{MT} was 0.41.

b. The relationship between m-RMT & EF_{MT} – Mono + CCD – Mono was evaluated. Together these two represent the amount of electric discharge delivered at a point in the cortex measured with reference to the center of the coil based on the model used by the neuronavigation system.
Together, EF_{MT} & CCD predicted m-RMT in 21 AD participants with an Adj. R^2 = 0.96 ($p \approx 10^{-13}$).
Taken together, these two results intuitively (and thus loosely) support the notion that RMT in the case of test cohort captured the probable cortical abnormality in AD.

All the above results in the case of biphasic RMT for the test cohort were qualitatively similar to those for monophasic RMT (shown here) & are presented in the supplementary information (ST-1,2, SF-1,2).

b. Validation Cohort

The association between RMT & cognitive decline prevails in a large AD cohort

Simple linear regression demonstrated a significant correlation between b-RMT & ADAS-Cog in this multi-center cohort of 128 AD participants (df = 1,126, β_{RMT} = - 0.07, Adj. R^2 = 0.07, p = 0.001) (Figure 3.3). Further, multiple regression models showed that neither age, gender or AD medication status. These results are summarized in Table 3.3. While reduction in the effect size and power of the relationship (as discussed ahead), compared to the one found in the test cohort is conspicuous, the fact that the correlation still prevails speaks to the utility of RMT in population-level clinical setting and draws attention to the problem of finding out the underlying neurophysiological alteration in AD being captured by RMT.

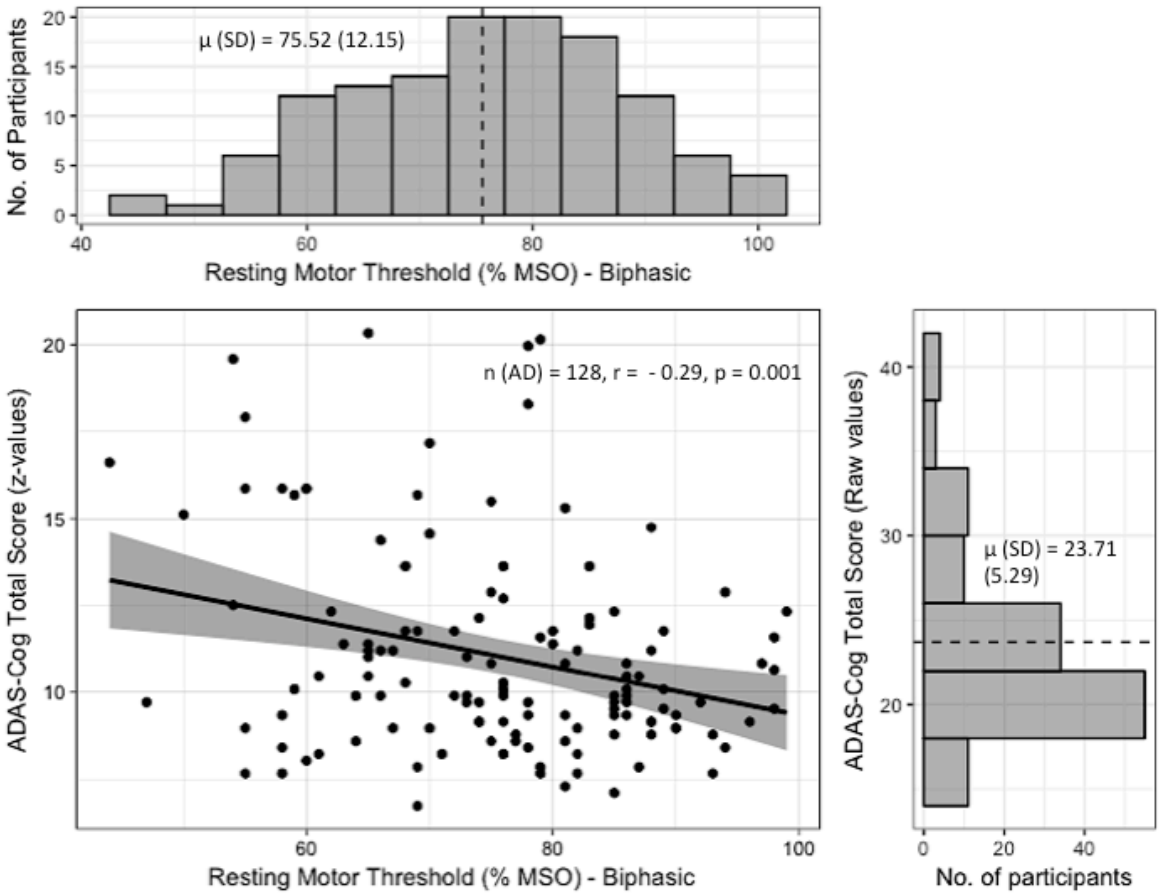

Figure 3.3: RMT – ADAS-Cog correlation in validation AD cohort – The scatter plot represents the correlation as found by simple linear regression (95% CI overlayed). The histograms in the side panels are the distributions of the raw values of both the variables, dotted line marking the distribution mean.

Model Regressors	df	β_{RMT}	% $\Delta\beta_{RMT}$	Adj. R^2_{model}	partial R^2_{covar}	p_{model}	p_{RMT}	p_{covar}
b-RMT	1,126	-0.07	-	0.07	-	0.001	0.001	-
b-RMT & Age	2,124	-0.07	0	0.09	0.03	0.003	0.003	0.18
b-RMT & Gender	2,125	-0.07	0	0.07	0	0.003	0.003	0.74
b-RMT & AD Medication Status	2,125	-0.07	0	0.09	0.03	0.003	0.003	0.18

Table 3.3: Multiple regression for RMT – ADAS-Cog relationship – The demographic covariates/factors were used in separate models to check their individual influence on the relationship. Partial R^2_{covar} measures the additional covariance explained by the covariate/factor. All p values for covariate models are corrected (green = highly significant p values).

c. Evaluation Cohort

MMSE and RMT are correlated in AD across studies

For the group (cohort) of nine studies considered here, a fixed-effects model resulted in a pooled correlation coefficient *r'* = 0.26 (95% CI = [0.16, 0.37], p < 0.0001). This value is closer in magnitude to the correlation value obtained for the validation cohort (cohort 2) from our study. The test of heterogeneity had a trending significance (Q = 18.08, p = 0.021). The random-effects model fitted using sampling variances as weights, resulted in a similar size of correlation with wider confidence intervals (*r'* = 0.26 [0.09, 0.44], p = 0.0032). A significant p-value along with the absence of null value in the 95% CI, reflect the statistical meaningfulness of the effect size. The test of heterogeneity had a trending significance (Q = 18.08, p = 0.021) with τ^2 = 0.025, where heterogeneity explained 47.3% (I2) of the total variability between studies (Figure 3.4).The mixed-effects model using demographic moderators predicted 64.58% of the estimated heterogeneity (k = 6, τ^2 = 0.01, I^2 = 18.72) while the individual moderators were not significant predictors of heterogeneity. The stimulation moderators model accounted for the 82.65% of the estimated heterogeneity (k = 9, τ^2 = 0.004, I^2 = 15.83), with RMT definition being a trendingly significant individual predictor (Z_b = - 2.11, p = 0.035). This holds in line with the *a priori* speculation that RMT definition might influence this correlation as it determines the extent of accuracy to which the underlying hyperexcitability is being measured in AD. A total model comprising of all the moderators taken together predicted 77.36% of the estimated heterogeneity (k = 6, τ^2 = 0.004, I^2 = 12.54). The results of the moderator models are not comparable among them due to the differences in the number of studies (k) included for their construction. This variation was due to unavailability of the data for moderators in some studies. The influence of individual moderators in the demographics & the total models might not have reached significance due to to the small sample size (k = 6). All the results are summarized in Table 3.4.

A.

Meta-analytic model	k (No. of studies)	Q (p-value)	τ^2 (SE)	I^2 (%)	H^2	Z - value (pooled *r'*)	Pooled *r'* (SE)	95% CI for *r'*	p
Fixed Effects Model	9	18.08 (0.021)	–	–	–	5.03	0.26 (0.05)	0.16, 0.37	< 0.0001
Random Effects Model	9	18.08 (0.021)	0.025 (0.02)	47.3	1.9	2.95	0.26 (0.09)	0.09, 0.44	0.0032

B.

Meta-regression models	k (No. of studies)	τ^2 (SE)	I^2 (%)	H^2	R^2 (%)	QE (df, p-value)	QM (df, p-value)
Mixed Effects - Demographic Moderators	6	0.01 (0.01)	18.72	1.23	64.58	8.02 (3, 0.046)	3.22 (2, 0.2)
Mixed Effects - Stimulation Moderators	9	0.004 (0.01)	15.83	1.19	82.65	10.58 (5, 0.06)	6.6 (3, 0.086)
Mixed Effects All Moderators	6	0.004 (0.008)	12.54	1.14	77.36	7.29 (2, 0.026)	3.87 (3, 0.276)

C.

Moderator	b (SE)	Z_b	p
Age	-0.11 (0.07)	-1.7	0.089
Gender	-0.05 (0.03)	-1.46	0.144

D.

Moderator	b (SE)	Z_b	p
Coil Shape	-0.54 (0.35)	-1.55	0.122
RMT Definition	-0.78 (0.37)	-2.11	0.035
Pulse Shape	0.95 (0.49)	1.95	0.051

Table 3.4: Meta-analysis & meta-regression of MMSE-RMT correlation – Results are summarized for the meta-analytic fixed- & random-effects models (panel A) as well as for mixed-effects models (panel B). Panels C & D are moderator effect summaries for demographic & stimulation moderator models respectively. (green – highly significant p-value, orange – borderline/trending significance).

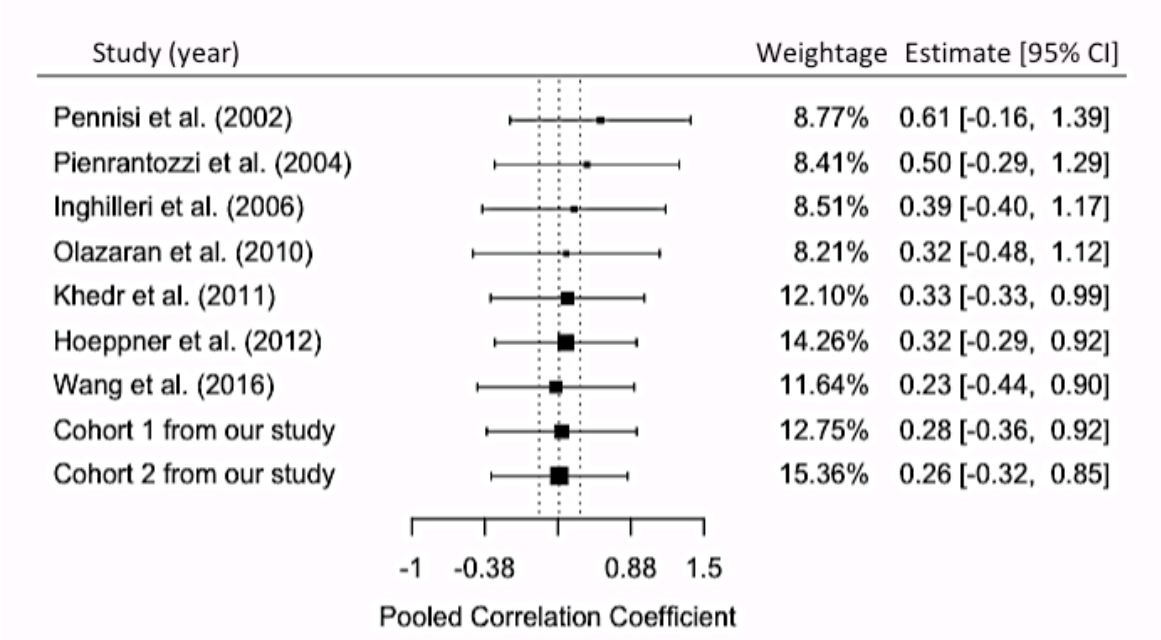

Figure 3.4: Forest plot for MMSE-RMT correlation meta-analysis – The forest plot summarizes the results of the random-effects model used here. The reference lines (vertical, dotted) mark the pooled correlation coefficient with 95% CI (r' = 0.26 [0.09, 0.44]). The corrected/standardized estimates of the individual correlations, along with their 95% CI are placed with reference to the total estimate. The marker size is proportional to the contribution of the study towards total estimate, given by weightage, here.

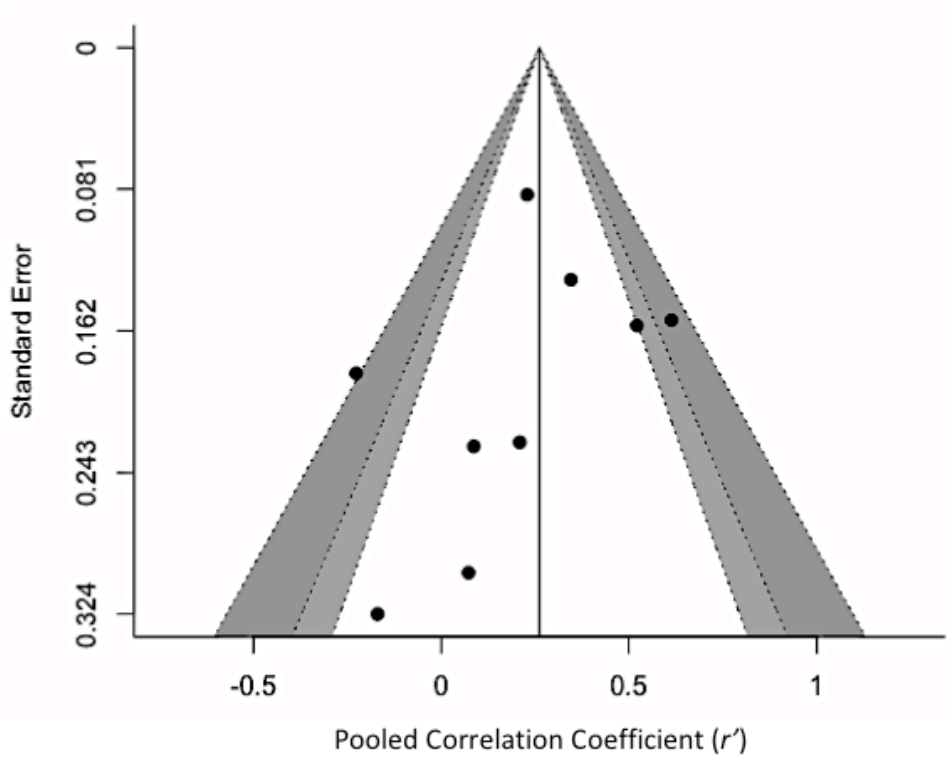

Figure 3.5: Funnel plot for MMSE-RMT meta-analysis – The plot is an indicator of the publication bias. The funnel is centered at the pooled correlation coefficient (r' = 0.26,

random-effects model) with the white, gray & dark gray regions marking the 90, 95 & 99% CI respectively. The asymmetry while visible is not starking in this case.

Further, the 95% prediction/credibility interval for the correlation coefficient calculated using random-effects model was – [- 0.09, 0.62]. This implies that based on the studies included in this analysis, a plausible value in this interval can be predicted for MMSE-RMT correlation in general. While the wide range of the credibility interval might not be useful for making predictions for the future studies, it creates a meaningful framework for placing the future results in the context of the current result, thereby improving our understanding of the true effect size of the correlation. A qualitative analysis of the publication bias is presented in the form of a funnel plot in Figure 3.5. The results of the analysis repeated after removing the data for our cohorts are given in the supplementary information (ST-3, SF-3,4).

3.2 Comparison of resting motor threshold (RMT) between healthy and dementia aging – RMT might be a group-level discriminator

For the group of 32 studies included in the analysis, a fixed-effects model resulted in a Hedge's G = - 0.65 (95% CI: [- 0.77, - 0.53], $p < 0.0001$) & was significant for the test of heterogeneity ($k = 32$, $Q = 249.22$, $p < 0.0001$). The weighted random-effects model fitting (Figure 3.6) gave a Hedge's G of - 0.91 (95% CI: [- 1.44, - 0.39], $p = 0.0006$). A significant p-value along with the absence of null value in the 95% CI, reflect the statistical meaningfulness of the effect size. Test for heterogeneity was statically significant ($k = 32$, $Q = 249.22$, $p < 0.0001$) & heterogeneity ($\tau^2 = 1.70$) accounted for 92.86% (I^2) of the total variability . This value falls in the high heterogeneity range. None of the demographic predictors (modeled separately) – age, gender, years of education & MMSE scores, were able to account for the estimated heterogeneity to a measurable amount. The model comprising of all the demographic moderators taken together couldn't account for any hetergeneity as well. The results of the moderator models are not comparable among them due to the differences in the number of studies (k) included for their construction. This variation was due to unavailability of the data for moderators in some studies. All the results are summarized in Table 3.5. Further, the prediction/credibility interval for the Hedge's G calculated using random-effects model was – [- 3.53, 1.70]. This implies

that based on the studies included in this analysis, a plausible value in this interval can be predicted for the standardized mean difference in general. Future studies validating/contradicting this result would help to understand the true effect size of the difference.

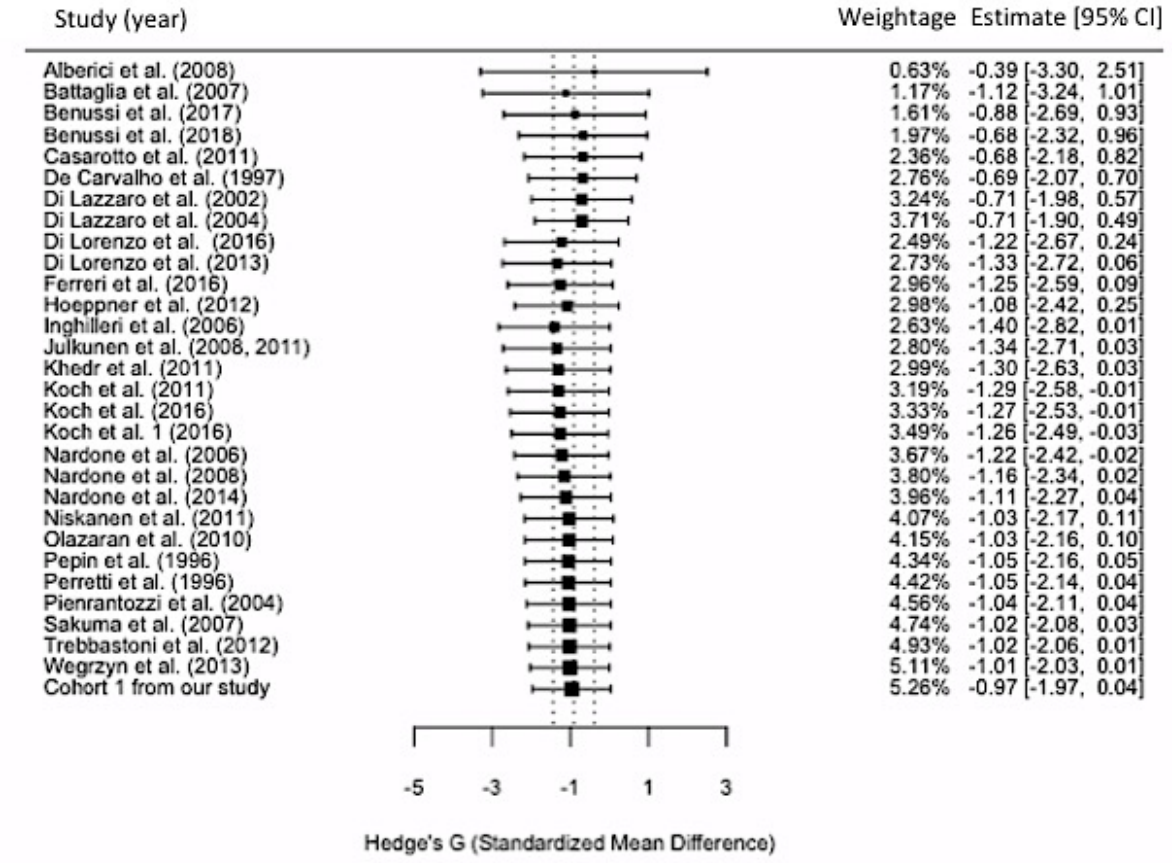

Figure 3.6: Forest plot for meta-analytic comparison between AD & CN RMTs – The forest plot summarizes the results of the random-effects model used here. The reference lines (vertical, dotted) mark the Hedge's G with 95% CI (g (SMD) = - 0.91 [- 1.44, - 0.39]). The corrected/standardized estimates of the individual SMDs, along with their 95% CI are placed with reference to the total estimate. The marker size is proportional to the contribution of the study towards total estimate, given by weightage, here.

The high heterogeneity which could not be accounted by demographic moderators could be due to biases in the research practice and/or publication bias among other reasons. This is reflected from the the funnel plot (Figure 3.7) which shows scatter of the points ranging wide beyond the confidence intervals. Further, the non-uniformity in the distribution of values along the Y-axis shows a bias towards publishing results that are highly significant in small sample sizes.

A.

Meta-analytic models	k (No. of studies)	Q (p-value)	τ^2 (SE)	I^2	H^2	Z - value (Hedge's G)	Hedge's G (SE)	95% CI for Hedge's G	p
Fixed Effects Model	32	249.22 (< 0.0001)	-	-	-	-10.23	- 0.65 (0.06)	- 0.77, - 0.53	< 0.0001
Random Effects Model	32	249.22 (< 0.0001)	1.70 (0.48)	92.86	14.02	-3.43	- 0.91 (0.27)	-1.44, - 0.39	0.0006

B.

Meta-regression models	k (No. of studies)	τ^2 (SE)	I^2	H^2	R^2	QE (df, p-value)	QM (df, p-value)
Mixed Effects - Age	31	1.81 (0.52)	93.29	14.91	0	246.96 (29, < 0.001)	1.58 (1, 0.21)
Mixed Effects - Gender	25	1.41 (0.47)	92.09	12.64	0	189.76 (23, < 0.001)	0.22 (1, 0.639)
Mixed Effects - Years of Education	14	1.75 (0.79)	92.18	12.79	0	109.76 (12, < 0.001)	0.94 (1, 0.333)
Mixed Effects - MMSE Scores	26	1.83 (0.58)	92.89	14.07	0	203.15 (24, < 0.001)	1.22 (1, 0.27)
Mixed Effects - Total	12	2.12 (1.2)	92.86	14.00	0	89.72 (7, < 0.001)	4.06 (4, 0.398)

C.

Moderator	b (SE)	Z_b	p
Age	0.14 (0.11)	1.26	0.209
Gender	0.01 (0.03)	0.47	0.639
Years of Education	0.13 (0.14)	0.97	0.333
MMSE Scores	0.16 (0.14)	1.10	0.270

D.

Total Mixed-Effects Model			
Moderator	b (SE)	Zb	p
Age	0.37 (0.22)	1.64	0.101
Gender	- 0.02 (0.06)	- 0.25	0.803
Years of Education	0.20 (0.17)	1.17	0.244
MMSE Scores	- 0.29 (0.33)	- 0.90	0.368

Table 3.4: Meta-analysis & meta-regression of RMT comparison between AD & CN – Results are summarized for the meta-analytic fixed- & random-effects models (panel A) as well as for mixed-effects models (panel B). Panels C summarises separately run models for demographic moderators. Total model results are in given in panel D. (green – highly significant p-value, orange – borderline/trending significance).

A qualitative analysis of the publication bias is presented in the form of a funnel plot in Figure 3.7.

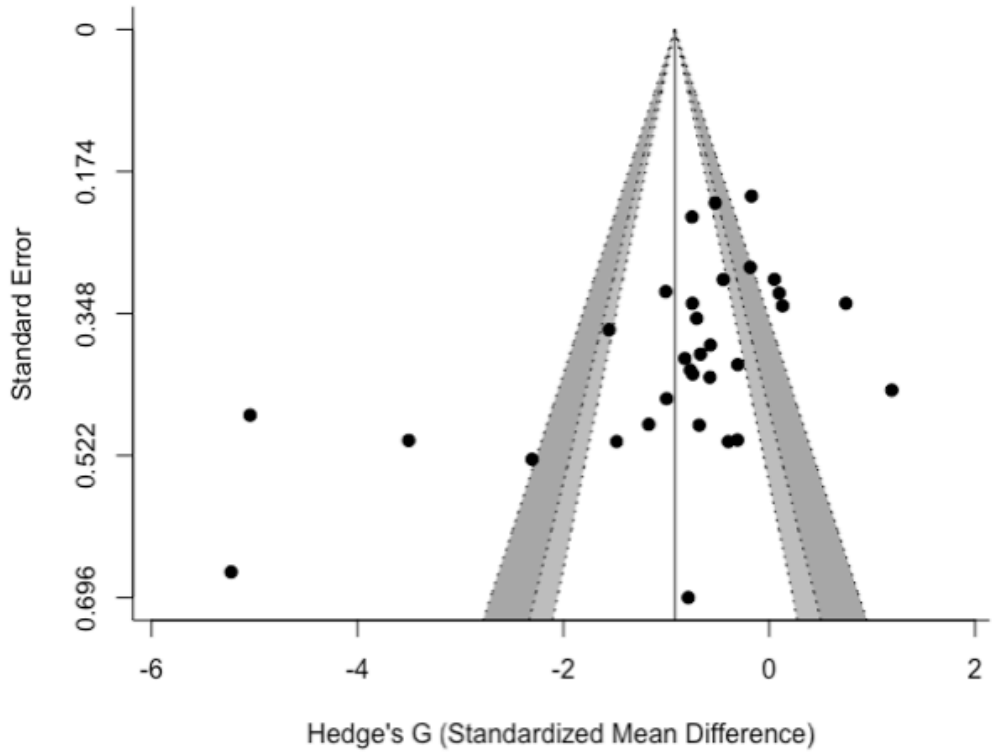

Figure 3.7: Funnel plot for MMSE-RMT meta-analysis – The plot is an indicator of the publication bias. The funnel is centered at the Hedge's G (g = - 0.91, random-effects model) with the white, gray & dark gray regions marking the 90, 95 & 99% CI respectively. The asymmetry is strikingly visible here with depreciation values towards the bottom of the funnel. Further, the spread of values beyond the CI shows bias towards highly significant –ve SMD values.

The overview of qualitative synthesis for all the studies used in both the meta-analyses is encapsulated in ST-4.

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4. Discussion

This thesis primarily presents the plausibility of using TMS resting motor threshold as a biomarker of cognitive decline in Alzheimer's disease, based on the neurophysiologically driven rationale that RMT measures the altered membrane/axonal excitability in AD.

In case of the test cohort, we have demonstrated that both monophasic and biphasic RMTs are significantly correlated with ADAS-Cog in the AD group but not in cognitively normal old people. The strength of correlation, however, appears to be slightly greater in the case of m-RMT ($r = - 0.62$, $p = 0.0038$) than that for b-RMT ($r = -0.60$, $p = 0.0056$). This could be due to Use of EF_{MT} as a substitute for RMT has previously been proposed by Nisskanen et al., 2011. Our analysis involving EF_{MT} provides a greater support for this notion. However, it should be noted that the E-field intensity calculations are based on models of the brain. If the models are not able to account for neurogenerative pathology (e.g., highly atrophied cortex or abnormal fibre tracks) involved in AD, then the inferences from EF_{MT} might be uninformative, if not inaccurate, about the underlying neurophysiology.

RMT, both monophasic & biphasic, did not differ significantly between AD & CN groups. However, the AD participants were significantly older compared to the CN participants ($p = 0.004$, uncorrected). Also, the hemispheric cortical thickness differed significantly between the two groups ($p < 0.001$, uncorrected). While both these factors can have an influence RMT, that did not seem to be the case with this cohort since the RMT difference between the two groups did not reach significance after controlling of these two factors (m-RMT – $p = 0.271$, uncorrected, b-RMT – $p = 0.296$). One possibility that can neither be tested not denied is that while the participants in the CN group were examined for cognitive intactness and underwent a neurological examination, the presence of prediabetes/ type-2 diabetes might have affected the resting motor thresholds for this group. Cortical excitability changes have been earlier reported in the conditions mentioned above previously.

A major limitation of the test cohort is the variability in the number of trials that were considered for CCD & EF_{MT} calculations. While ideally, the total number of trials contributing to all RMT related measurements should be between 5 & 10, TMS operators may have conducted & considered a varying number of trials depending on adjustments (due to shift in coil position, tilt, etc.) in the stimulation spot in between the trials, for ensuring the comfort of the participants, any unanticipated interruptions in the stimulation sequence or any other reasons of pragmatic difficulty. While we had no control over the collection of these data values, to prevent selection bias, the average of all the trials in a sequence of MEP measurements, for which the %MSO was equal to RMT was considered for calculating EF_{MT} and CCD.
Reduction in the predictability and significance for the validation cohort results can be due to several reasons. For this cohort, the RMT was measured in the absence of EMG. It has been shown previously that visual observation of muscle twitch is less accurate than EMG, leading to higher variability in measurements and significant overestimation. This is likely because the MEPs of low amplitude that are above 50 μV but that do not cause 'muscle twitch,' get discarded. Another possible reason for the weaker relationship in this cohort could be the absence of neuronavigation. Neuronavigation improves the search for motor hotspot and increases the precision of targeting the appropriate stimulation spot by several folds. Against these major drawbacks, we were able to find a significant relationship between RMT & ADAS-Cog in a heterogeneous AD population tested across multiple operators, in the absence of EMG & neuronavigation. We believe that this makes a case towards the greater utility of RMT as a marker of cognitive decline in AD in a population level clinical setting.

The results from the meta-analysis (and the meta-regression) of the MMSE-RMT correlation provided further support to the notion of cortical excitability being related to the cognitive in AD. A pooled correlation value closer to the one found in the case of our validation cohort, with a moderate heterogeneity between studies which was further explained by demographic and stimulation-dependent moderators increases the credence of our notion. To check if the data from our cohorts is biasing the results, the models were re-run after removing the cohort 1 & cohort 2 from our study. This however gave qualitatively similar results for the random-effects model: pooled correlation r' = 0.23 (95% CI: [0.0025, 0.46], p = 0.0475) (details described in

supplementary information). The lowering of the strength of correlation and the reduction in significance does not necessarily reflect the influence of data from our cohorts, as these could also be due to the worse fitting of the model to the smaller size of the data (k = 7 instead of 9). This can be observed in the over-fitting of the mixed-effects models for this analysis, predicting 100% of the between-study heterogeneity (supplementary information). Hence, the inclusion of data from our cohorts, probably, does more towards strengthening the results than biasing them. Viewing the meta-analysis results in the context of results from the test & the validation cohorts, an important consideration is a cognitive test being used for the correlation. ADAS-Cog is known to be more comprehensive, more sensitive and specific measure of cognitive function (or dysfunction) compared to MMSE. Hence, a meta-analysis involving ADAS-Cog might give a better estimate of the correlation with RMT than that inferred from MMSE here. As mentioned earlier unavailability of data impeded us from conducting such analysis. Hence, future studies collecting and reporting extensive neuropsychological data would help such meta-analytic studies.

A qualitative comparison of AD and CN groups for their resting motor thresholds have been presented earlier in several systematic reviews exploring cortical hyperexcitability in AD (Freitas et al., 2011) (Benussi et al., 2018). The meta-analysis presented here is the first attempt towards a quantitative assessment of this problem. While our analysis reveals an estimated difference between AD & CN – RMTs that reaches statistical significance & is similar in size to earlier reports, the high level of heterogeneity between studies impedes drawing useful inferences. One major reason could be that the AD participants across studies vary regarding their disease stages. We attempted to assess & account for this using MMSE scores as a moderator, but the residual heterogeneity was still in the higher range. Our meta-regression analysis using other demographic moderators could not account for the most of the heterogeneity as well. The risk of publication bias, as revealed by the funnel plot (Figure 3.7) could then be a major driving reason. Using compound stimulation scores as weights was an attempt to reduce the problem of publication bias towards positive results based on data of differing quality standards. Since a large number of selected studies share more than one authors, the risk of bias, e.g., the unreported overlap of study subjects across publications, etc. cannot be denied. Bibliometric & co-citation network analysis might help understand this problem better.

Our results make a case for the possibility of RMT acting as a discriminant between AD & CN groups, but these should be regarded with caution. Due to data unavailability, we could perform an analysis (such as a sensitivity analysis) to determine if RMT can discriminate between AD & CN at the level of an individual. However, given the inter-individual variability of RMT measurements, its value as a diagnostic marker is doubtful.

Considering that this was an analysis of the retrospective cross-sectional data, a definitive comment on the use of RMT as a diagnostic marker of AD or a predictive marker for the cognitive decline in AD is beyond the scope of this thesis. However, we believe that this thesis sets a crucial premise for future investigations in those directions. We believe that this study opens doors on two sides. On the research side, prospective studies should test the proposed notion of altered neuronal membrane/axon excitability, and it's contribution to the total hyperexcitability in AD. This question can be answered at various levels. AD animal models can use to understand the viability and mechanistic details of our proposition. In case of human translational studies, another test of our proposal would be to assess the relationship between cognitive decline & excitability threshold measured using TMS-EEG in non-motor areas known to be involved in a specific domain of the cognitive function, such as DLPFC (dorsolateral prefrontal cortex). This will help understand the problem nudged in our exploratory analysis & also provide insight into the proposed rationale. TMS-EEG can be useful for yet another purpose. We have demonstrated that the relationship between RMT and cognitive decline is driven by the contribution of the cortical excitability towards RMT, and not the spinal component, by evaluating the role of EF_{MT}. Another way to evaluate the issue of non-cortical contributions to hyperexcitability is TMS-EEG. TMS evoked potentials (TEPs) as measured by EEG might provide greater evidence for strengthening the notion of cortical excitability in place of corticospinal excitability.

On the clinical side, the biomarker utility of RMT is supported by several pragmatic advantages. TMS being non-invasive is a more feasible marker for multiple measurements over several follow-ups, compared to PET (e.g., Tau-PET) that depends on radioactive agents. With regards to the underlying 'substrate' of analysis, RMT is represented a specific region/circuit in the cortex compared to global maps of

glucose hypometabolism provided by FDG-PET or the brain atrophy measures (changes in volumes or thickness) deciphered from MRI. A fair counter-argument can be supported by the availability of advanced image-processing techniques that can provide information about localization of neuropathology to a high resolution. However, we find it crucial to stress here that these techniques are intensive with regards to human & economic resources. Hence, their clinical utility in the immediate future is questionable. Further, variability in single slice-time MRI assessments impedes useful inferences in clinical cases. The comparative economic advantage of TMS equipment, as well as the TMS visit costs over PET or MRI, also needs to be taken into account. Neuropsychological testing is currently the most sensitive measure of cognitive decline. However, it is time-consuming, and human resource intensive since highly trained personnel is required for conducting and scoring the tests. Moreover, the testing depends on the active participation of the patient, which becomes increasingly difficult in AD as the disease progresses. RMT can be undertaken in a resting individual by a trained professional within minutes without requiring any active involvement of the patient or any post-measurement scoring. Currently, RMT measurements cannot replace neuropsychological testing, but their addition to the test visits might be useful. RMTs exhibit wide inter-individual variability, thus portraying the potential of being utilized as individualistic markers. However, intra-individual test-retest reliability for RMT over short intervals has been reported to be highest among all TMS measures of cortical excitability & plasticity. Taken together this means that changes in RMT across long timescales can act as reliable markers of changes in the underlying neurophysiology, ruling out variability due to inconsistency across measurements (Fried et al., 2017). Future studies should exploit these features of RMT in a longitudinal comparative (AD vs. CN) cohort design to assess the utility of RMT as monitoring and more importantly as a predictive biomarker for cognitive decline in AD progression. Longitudinal studies will further provide insight into relative placement of RMT in the current biomarker models of AD. One of the most exciting applications of RMT following directly from our rationale & having clinically useful implications would be the pharmacodynamic biomarker value of RMT in treatment measures involving pathological ion channels, membrane-embedded oligomers, etc. as their targets.

In conclusion, this thesis attempts to make an original contribution for making a case

for TMS-RMT as a biomarker of AD. In first ever, we present a neurobiological rationale for abnormal RMTs. We test this rationale in greatest possible research detail & establish it's utility in a clinical setting. Our meta-analytic studies not only summarize the corroborative evidence from the past but also provide directions to the future studies.

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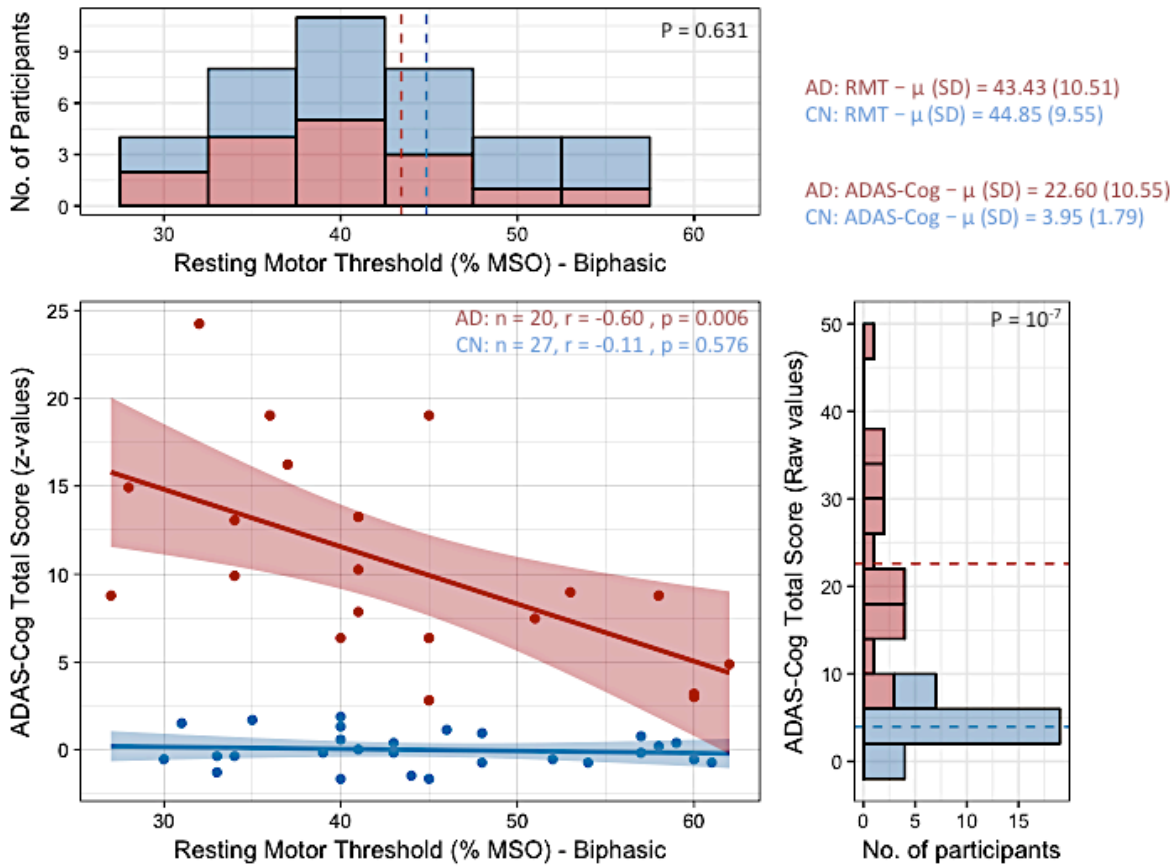
6. Supplementary Information

Test Cohort ADAS-Cog – b-RMT Analysis

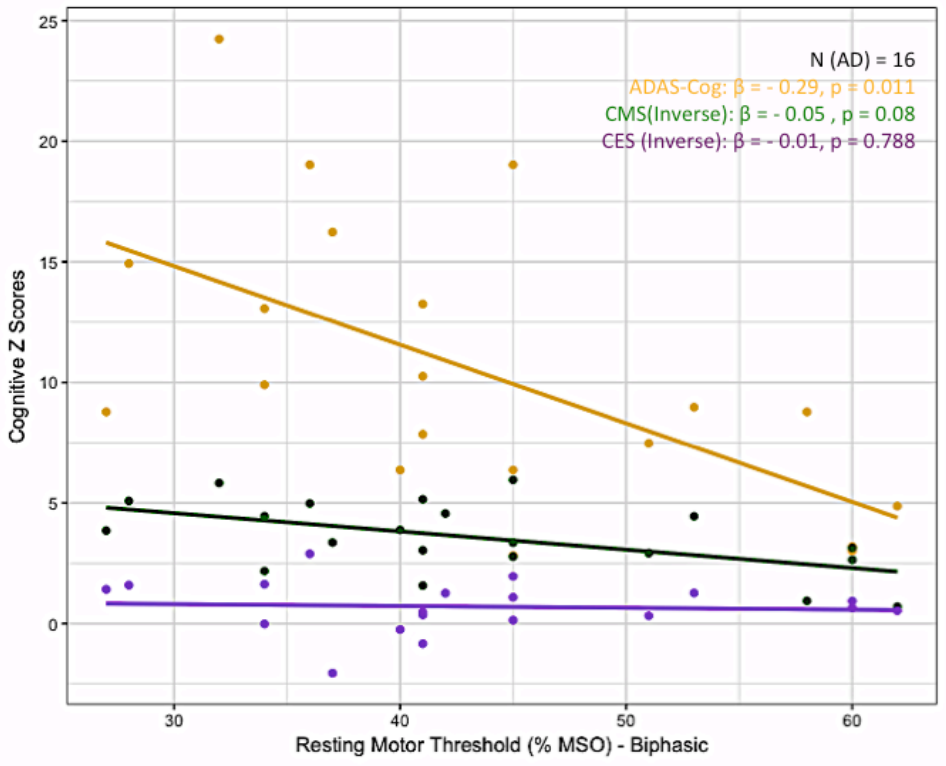


SF-1: Correlation between b-RMT & ADAS-Cog in test cohort – The scatterplot represents the correlation between ADAS-Cog (z- values) and Biphasic RMT as found by simple linear regression (95% CI provided) for AD (red) & CN (blue) groups. The histograms in the side panels provide the distributions of the raw values for both variables with a comparison of how the AD & the CN groups differ for them (uncorrected p -values).

Model Regressors	df	β_{RMT}	% $\Delta\beta_{RMT}$	Adj. R^2_{model}	partial R^2_{covar}	p_{model}	p_{RMT}	p_{covar}
b-RMT	1,18	-0.33	-	0.32	-	0.006	0.006	-
b-RMT & Age	2,17	-0.34	-3.03	0.3	0.03	0.042	0.04	n.s
b-RMT & Gender	2,17	-0.31	6.06	0.33	0.07	0.042	0.049	n.s
b-RMT & Hand-	2,16	-0.33	0	0.26	0	0.042	0.055	n.s

dominance								
b-RMT & ApoE- ε4	2,9	-0.34	-3.03	0.23	0.31	0.072	0.102	n.s
b-RMT & BDNF- Met	2,9	-0.34	-3.03	0.34	0.41	0.072	0.102	n.s
b-RMT & CCD - Bi	2,17	-0.33	0	0.28	0	0.042	0.049	n.s
b-RMT & SCD	2,17	-0.35	-6.06	0.34	0.08	0.04	0.036	n.s
b-RMT & CT (LH)	2,17	-0.27	18.18	0.38	0.14	0.036	0.072	n.s
b-RMT & EFMT - Bi	2,17	-0.33	0	0.28	0	0.042	0.102	n.s

ST-1: Multiple regression for b-RMT – ADAS-Cog relationship in AD –Covariates/factors were used in separate models to check their individual influence on the relationship. The % Δβ > 10 is an indirect measure of greater influence (orange). Partial R^2_{covar} measures the additional covariance explained by the covariate/factor. All p values for covariate models are corrected (green = highly significant p values).



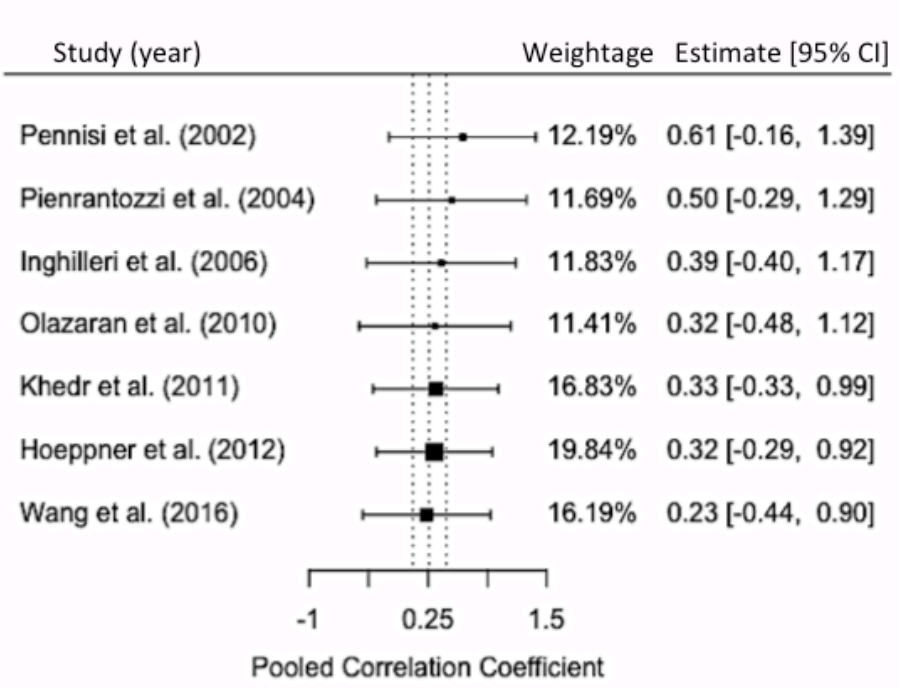
SF-2: b-RMT correlations across different cognitive test measures – Univariate statistics summary from MANOVA gives individual correlations for b-RMT with ADAS-Cog, CMS & CES. For better representation, the CMS & CES scores are inverted. Hence, for all tests, a higher Z score here represents greater dysfunction.

	df	Pillai's Test Statistic	F (approx)	p value
MANOVA results	3.14	0.35	2.51	0.101

Cognitive Score	df	β_{RMT}	Adj. R^2_{model}	F-statistic	p_{model}
ADAS-Cog	1,16	-0.29	0.3	8.22	0.011
CMS	1,16	0.05	0.13	3.45	0.08
CES	1,16	0.01	-0.06	0.07	0.788

ST-2: MANOVA results & univariate summaries for biphasic RMT with ADAS-Cog, CMS & CES as dependent variables.

MMSE-RMT Correlation Meta-analysis for only the published studies (k = 7)



SF-3: Forest plot for MMSE-RMT correlation meta-analysis (k = 7) – The forest plot summarizes the results of the random-effects model used here. The reference lines (vertical, dotted) mark the pooled correlation coefficient with 95% CI (r' = 0.23 [0.0025, 0.46]). The corrected/standardized estimates of the individual correlations, along with their 95% CI are

placed with reference to the total estimate. The marker size is proportional to the contribution of the study towards total estimate, given by weightage, here.

A.

Meta-analytic models	k (No. of studies)	Q (p-value)	τ^2 (SE)	I^2	H^2	Z - value (pooled *r'*)	Pooled *r'* (SE)	95% CI for *r'*	p
Fixed Effects Model	7	15.09 (0.0196)	-	-	-	3.17	0.23 (0.07)	0.09, 0.38	0.0015
Random Effects Model	7	15.09 (0.0196)	0.04 (0.04)	52.10	2.09	1.98	0.23 (0.12)	0.0025, 0.46	0.0475

B.

Meta-regression models	k (No. of studies)	τ^2 (SE)	I^2	H^2	R^2	QE (df, p-value)	QM (df, p-value)
Mixed Effects - Demographic Moderators	4	0	0	1	100	0.09 (1, 0.77)	7.22 (2, 0.027)
Mixed Effects - Stimulation Moderators	7	0	0	1	100	6.44 (3, 0.092)	8.65 (3, 0.034)
Mixed Effects All Moderators	6	0.004 (0.008)	12.54	1.14	77.36	7.29 (2, 0.026)	3.872 (3, 0.276)

C.

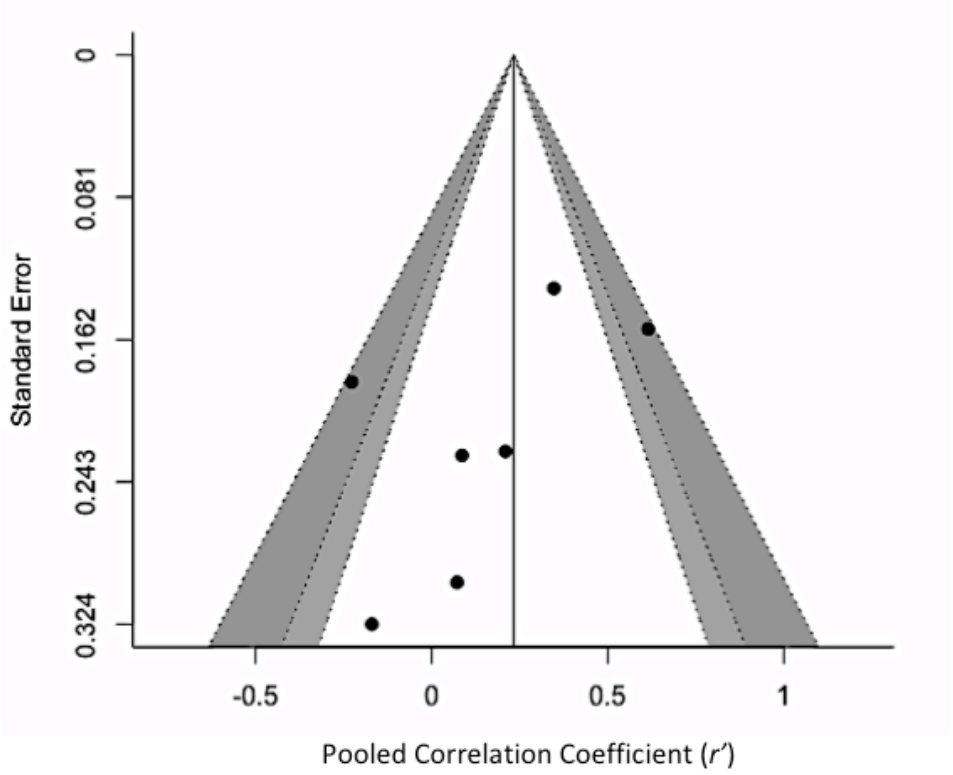
Moderator	b (SE)	Z_b	p
Age	0.03 (0.08)	0.31	0.756
Gender	0.08 (0.06)	1.44	0.148

D.

Moderator	b (SE)	Z_b	p
Coil Shape	-0.78 (0.36)	-2.182	0.029

RMT Definition	0.86 (0.48)	1.812	0.07
Pulse Shape	-0.54 (0.34)	-1.604	0.109

ST-3: Meta-analysis & meta-regression of MMSE-RMT correlation (k = 7) – Resullts are summarized for the meta-analytic fixed- & random-effects models (panel A) as well as for mixed-effects models (panel B). Panels C & D are moderator effect summaries for demographic & stimuation moderator models respectively. (green – highly significant p value, orange – borderline/trending significance).



SF-4: Funnel plot for MMSE-RMT meta-analysis (k = 7) – The plot is an indicator of the publication bias. The funnel is centered at the pooled correlation coefficient (r' = 0.23, random-effects model) with the white, gray & dark gray regions marking the 90, 95 & 99% CI respectively. The asymmetry while visible is not starking in this case.

Study Information		Demographic Information						Clinical, Neurological, Neuropsychiatric, and Neuropsychological Information			TMS Related Information						Measures of Cortical Excitability		RMT Definition	Group Comparison			Correlation with Cognition					
Article	Group	N	Gender Ratio (M/F) or %F	Age mean ± SD or [range]		Education mean ± SD		Disease Duration in yrs, (SD), [range]	Disease Stage	Neuropsych testing mean, (SD), [range]	Stimulation Parameters	EMG Status	Imaging	Neuronavigation Status	Stimulation Site	Muscle recorded	Resting Motor Threshold (RMT)		Definition of RMT (minimum uV, #trials)	Test for comparison	Significance	Conclusion [1]	Neuropsychological test used	Method of correlation	correlation value (r)	Significance of the relationship	Conclusion	Meds?
				Mean	SD	Mean	SD										Mean	SD										
Olazaran et al. (2010)	AD	11	5/6	77.2	4.4	9 participants had no/incomplete edcuation, but all were literate. 2 participants had ≥primary education.		2.7 (±1.9)	very mild AD	MMSE 21.9 (±3.1); CDR 0.5; ADAS-Cog 19.3 (±4.4)	circular coil, two magstim 200 stimulators connected via BiStim module	-	CT	-	L M1	FDI	50	9.3	50 uV, 3/6 trials	-	0.42	not significantly different between groups	-	-	-	-	-	yes meds - donepezil
	HC	12	6/6	77	5	6 participants had no/incomplete education, but all were literate. 6 participants had ≥primary education.		-	-	MMSE 27.3 (±2.5); ADAS-Cog 9.7 (±3.9)							55.8	10.1										
Hoeppner et al. (2012)	AD	19	7/12	71.8	4.7	-	-	-	probable AD	MMSE 21.7 (±4.9), [10-27]	figure-8 shaped coil, MagPro 100, MagOption Medtronic, placed tangentially oriented in mediolateral direction	high-gain display of EMG amplifier signals	-	-	L M1	FDI	59.21	7.57	50 uV, 6/10 trials	One-way ANOVA	0.024	AD group had significantly lower RMT	MMSE	Pearson	MMSE and TMS r = 0.209	not significiant	no correlation between MMSE and TMS	none
	HC	19	9/10	69.2	3.7	-	-	-	-	MMSE 28.6 (±1.0), [26-30]							52.95	8.82										
Khedr et al. (2011)	AD	45	16/29	68.4	55-82 [range]	25 participants were illiterate, 5 could read and write, 11 attended primary or secondary school, 3 attended university		3.7 (±2.5), [.5-10]	12 mild, 21 moderate, 12 severe	MMSE 14.8 (±5.5), [4-23]; CDR 2.24 (±0.76)	figure-8 shaped coil, MagStim super rapid, external loop 90mm, placed tangentially, oriented perpendicular to the central sulcus at 45 degree angle from midsagittal line		CT, MRI	-	both R and L M1	FDI	39.5	6.5	50 uV, 5/10 trials	One-way ANOVA	0.0001	AD group had significantly lower RMT	MMSE, CDR	ANOVA?	no R values found, p values found	significant for both tests	sig positive correlation between MMSE and RMT (p=0.009) , lower MMSE, lower RMT	none
	HC	37	-	65.7	6.7, 55-81 [range]	23 participants were illiterate, 5 could read and write, 7 attended primary or secondary school, 2 attended university		-	-	MMSE 27.1 (±1.1) [25-28]							44	5.2									sig negative correlation between CDR and RMT (p=0.01)	
Pennisi et al. (2002)	AD	17	7/10	74 [median]	55-82 [range]	-	-	-	probable AD	MMSE 11.76 (±5.68)	circular coil, Magstim 200, diameter 9 cm, lateral to the hemisphere	electrodes, Medelec Premier, gains - 100 uV, 5 mV/div, band pass - 30 Hz - 3kHz	CT, MRI	-	R M1, L M1	FDI	LH: 37.94; RH: 38.41	LH: 9.41; RH: 10.02	20uV ,50% of 10-20 trials	-	-	-	MMSE					none
	HC	-	-	-	-	-	-	-	-	-																	
Pienrantozzi et al. (2004)	AD	12	-	65.2	3.5	-	-	<1.5 years	mild	MMSE 21.8 (±2.1)	figure-8 shaped coil, Magstim 200 (MSO 2.2 T), diameter 70mm, biphasic, 45 degrees to midline	belly tendon montage, impedence < 5kohm, filtering bandwidth 2 -2000 Hz, sampling rate 5kHZ on 200 ms sweep duration	MRI, 99 HMPAO SPECT	-	R M1, L M1	APB	LH: 56.6; RH: 59.3	LH: 7.5; RH: 8.3	100 uV, 3/6 trials	Two-way ANOVA	p value not given	not significantly different between groups	MMSE					none
	HC	12	-	64.5	3.2	-	-	-	-	-							LH: 58.9; RH: 58.7	LH: 7.1; RH: 7.4									
Inghilleri et al. (2006)	AD	20	-	71	2	-	-	3.7 (±0.3)	mild to moderate	MMSE 16; ADAS-Cog 39	figure-8 shaped coil, Magstim Rapid, biphasic, 45 degrees to midline	Digitimeter D360, bandwidth 20Hz - 1kHz	MRI, CT	-	L M1	FDI	54.4	1.74	50 uV, 5/10 trials	One-way ANOVA	p = 0.0004	AD group had significantly lower RMT	MMSE					none
	HC	20	-	69	1.6	-	-	-	-	-							64.4	2									
Perretti et al. (1996)	AD	15	4/11	67.2	7.8, 58-80 [range]	7.3	4.5	"progressive decline in intellectural activities for at least 1 year"	7 moderate; 8 moderately severe	MMSE moderate 21.7 (±3); MMSE severe 13.7 (±5.6)	magnetic stimulator DANTEC MAG 2 with monophasic pulse. a flat circular coil	electromyography via surface electrodes	MRI, CT	-	the coil was moved over the scalp vertex to localize the optimal stimulation site for eliciting threshold motor responss in APB	APB	68.3	17.7	3 responses out of 6 of an amplitude >20uv	paired and unpaired t-test, ANOVA	p<0.02	MEP threshold intensity in the APB muscle was increased in 6 patients, 5 o whom were at the more advanced stages of the disease. The other 9 patients showed normal MEP						no meds
	HC	10	3/7	67.8	5, 57-73 [range]	-	-	-	-	MMSE							49.3	11									
Pepin et al. (1996)	AD	17	4/13	70.7	6.6, 57-86 [range]	-	-	-	6 mild, 8 moderate, 3 moderately severe	-	figure-8 shaped, Magstim 200	EMG signals were recorded through an analogue/digital interface CED 1401plus	-	-	L M1	ADM	34.9	6.5	50uv in 5 consecutive trials	paired and unpaired t-tests, ANOVA	p<0.001	AD group had significantly higher RMT						
	HC	old: 22 young:29	old: 6/16 young:17/12	old: 68.5 young: 34.1	old: 9.2, 51-81 [range] young:34.1, 22-48 [range]	-	-	-	-	-							old: 49.7 young: 46.6	old: 11 young: 6.1									
Sakuma et al. (2007)	AD	12	-	-	-	-	-	-	-	MMSE 21.8 (±2.8)	figure-8 shaped, high power Magstim 200	Micro 1401; Cambrigde Electronic Design	MRI	-	L M1	FDI	46.83	8.11	50uv	ANOVA	p<0.05 was used as the significant threshold	no significant different between groups	-	-	-	-	-	none
	HC	15	-	-	-	-	-	-	-	-							53	9.6									
Julkunen et al. (2008)	AD	5	40% F	73.2	8.1	9.6	2.1	-	mild	MMSE 22 (±5.1); CDR 0.6 (±0.2)	figure-8 shaped, Magstim 200, monophasic	continuous electromiography (ME 6000, Mega Electronics Ltd)	MRI	Nexstim	R M1, L M1	thenar and hypothenar muscle	LH: 41.6; RH: 40.7	LH:4; RH 5.6	50uV, 4/10 trials	non-parametric Mann-Whitney test	p<0.05	controls, but not different from that of MCI. In addition, MCI also showed a significant decrease in the N100 amplitude compared to controls.	-	-	-	-	-	AD: cholinesterase inhibitors
	HC	4	75% F	77.8	2.6	6.5	1	-	-	MMSE 27 (±4.1); CDR 0.0 (±0.0)							LH: 48.4; RH: 48.4	LH:10.8; RH 10.9									
Nardone et al. (2008)	AD	17	10/7	68.4	58-74 [range]	-	-	-	very mild, or mild	CDR	figure-8 shaped, Magstim 200	Motor responses were amplified and filtered by D150 amplifiers	-	-	Dominant hemisphere	FDI	50.6	11.6	50 uV, 5/10 trials	Mann-Whitney tests	p>0.05	no significant different between groups	-	-	-	-	-	none
	HC	22	12/10	70.4	62-73 [range]	-	-	-	-	-							49.5	10.8									

Study Information		Demographic Information						Clinical, Neurological, Neuropsychiatric, and Neuropsychological Information			TMS Related Information						Measures of Cortical Excitability		RMT Definition	Group Comparison			Correlation with Cognition					
Article	Group	N	Gender Ratio (M/F) or %F	Age mean ± SD or [range]		Education mean ± SD		Disease Duration in yrs, (SD), [range]	Disease Stage	Neuropsych testing mean, (SD), [range]	Stimulation Parameters	EMG Status	Imaging	Neuronavigation Status	Stimulation Site	Muscle recorded	Resting Motor Threshold (RMT)		Definition of RMT (minimum uV, #trials)	Test for comparison	Significance	Conclusion [1]	Neuropsychological test used	Method of correlation	correlation value (r)	Significance of the relationship	Conclusion	Meds?
Julkunen et al. (2011)	AD	5	3/2	73.2	8.1	9.6	2.1	-	mild	MMSE 22 (±5.1); CDR 0.6 (±0.2)	figure-8 shaped, Magstim BiStim	continuous electromiography (ME 6000, Mega Electronics Ltd)	MRI	Nexstim	R M1, L M1	thenar and hypothenar muscles	LH 48.4; RH: 40.2	LH: 10.8; RH: 10.9	50uV, 4/10 trials	Mann-Whitney tests	p<0.05	right hemisphere of the control subjects was significantly lower than in MCI. the TEP P30 was significantly lower in AD compared to controls, but not different from that of MCI	MMSE, CDR	Spearman's rank correlation	no r values	MMSE= p<0.05; CDR= p<0.01; CDR-SOB= p<0.001	an inverse correlation was found between the global CDR and P30 amplitude, as well as between CDR-SOB and P30 amplitude. A positive correlation was observed between MMSE and P30	AD: cholinesterase inhibitors
	HC	4	1/3	77.8	2.6	6.5	1	-	-	MMSE 27 (±4.1); CDR 0.0 (±0.0)														no r values			
Niskanen et al. (2011)	AD	15	5/10	73.7	7.5	-	-	-	-	MMSE 18.9 (±4.1)	figure-8 shaped, Nexstim, biphasic	ME 6000 Mega Electronics Ltd. for continuous recording	MRI	eXimia	hand knob	APB	89.7	17.4	50 uV, 5/10 trials	ANCOVA	p<0.05	sensorimotor cortex counteracting the prominent loss of neurons since that cortical excitability was found to do not correlate with cortical thinckeness.	-	-	-	-	-	12 out of 15 AD patients were under AChEI medication
	HC	21	10/11	71.9	5.9	-	-	-	-	MMSE 28.4 (±1.4)							87.8	11.9									
Trebbastoni et al. (2012)	AD	11	3/8	78	1.4	-	-	> 1 month	mild to moderate	MMSE	figure-8 shaped, Magstim Rapid, biphasic	Micromed Brain Quick	MRI	only 4 subjects had MRI	-	L M1	57.4	1.6	50 uV, 5/10 trials	ANOVA	p<0.05	AD group had significantly lower RMT	MMSE, ADL and IADL	Pearson's	no r values	p>0.05	no significant correlation was found between patients neuropsychological scores and TMS measures.	no meds at enrollement, but between the 1st and second rTMS sessions (30 days apart) they were treated with Rivastigmine
	HC	11	4/7	75.2	1.3	-	-	-	-	MMSE 28.7						FDI	64.4	2.4									no meds
Terranova et al. (2013)	AD	10	6/4	79.7	5.1	-	-	2.6 (±1.4)	mild	MMSE 22.7 (±1.25); CDR 1 (±0.0)	figure-8 shaped, Magstim Rapid, 9cm diameter	present, DIGITIMER D 150 amplifier	-	-	R M1	APB	42.5	-	50 uV, 5/10 trials	Factorial ANOVA	p<0.05	AD group had significantly lower RMT at baseline	-	-	-	-	-	no meds
	HC	14	9/5	77	7.1	-	-	-	-	-							59.2	-									
Wegrzyn et al. (2013)	AD	19	7/12	71.8	4.7	11	2.8	-	mild, moderate, and severe	MMSE 21.7 (±4.9), [10-27]	figure-8 shaped, MagPro 100	display of EMG amplifier signals and auditory feedback. "Brain Vision Amplifier" MES	1.5 MRI (T1 & T2), DTI	-	L M1	FDI	52.95	8.8	50 uV, 6/10 trials	T-test	p<0.05	AD group had significantly lower RMT	-	-	-	-	-	-
	HC	19	9/10	69.2	3.7	14.1	2.4	-	-	MMSE 28.6 (±1), [26-30]							59.21	7.6									
Nardone et al. (2014)	AD	8	-	72.5	6.1	-	-	-	-	MMSE	figure-8 shaped, high power Magstim 200, external loop 70 mm	D150 amplifiers were used (Digitimer, UK)	MRI	-	Dominant hemisphere	FDI	43.85	12.4	50 uV, 5/10 trials	Two-way ANOVA	no p reported, but presumably p>0.05	AD group had lower RMT, but not significant	-	-	-	-	-	none
	HC	8	-	72.5	6.1	-	-	-	-	-							48.13	13.91									
Wang et al. (2016)	AD	27	12/15	72.07	1.62	10.96	0.77	-	mild to moderate	MMSE 19.85 (±0.73); CDR "all patients scored between 1 and 2"	butterfly shaped, MagVenture Magilite Pro 30, loop diameter 50mm	yes, with acoustic feedback	-	-	R M1, L M1	APB	LH: 43.96; RH: 44.77	LH:1.34; RH:1.44	50 uV, 5/10 trials	T-test	p=0.007	AD RMT was significantly lower compared to FTD	MMSE, MoCA	Pearson's correlation	MMSE: r=-0.227, P=0.264; MoCA: r=-0.063, P=0.758	no significant	there was no correlation between MMSE/MoCA and RMT	none
	-	-	-	-	-	-	-	-	-	-							-	-									
Di Lazzaro et al. (2004)	AD	28	-	71.3	6.8	8.23	2.3	2.67 (±1.4)	probable AD	MMSE 19.35 (±3.8), [9-28]	figure-8 shaped, two high power Magstim 200s connected to BiStim module, diameter 9cm, posterior --> anterior direction, held over right motor cortex at the optimum scalp position	D150 amplifiers (digitimer UK)	-	-	R M1	FDI	49.9	10.9	50 uV, 5/10 trials	Mann- whitney test	p<0.05	AD group had significantly lower RMT	-	-	-	-	-	to test if RMT was sensitive to changes in ACh activity, MT was examined in 14 pts before and after 3mg of rivastigmine (separate from main focus of study), not significant
	HC	12	-	73.1	5.4	-	-	-	-	MMSE >23.7							57.9	11.7									
Di Lazzaro et al. (2002)	AD	15	9/6	69	5.3	9.3	3.4	2.03 (±1.21)	probable AD	MMSE 18.6 (±3.5)	figure-8 shaped, high power Magstim 200, 9cm diameter, posterior-->anterior direction, held at optimum scalp position	-	-	-	R M1	FDI	50.2	6.4	50 uV, 5/10 trials	Unpaired t-test	p<0.05	AD group had significantly lower RMT	-	-	-	-	-	to test if RMT was sensitive to changes in ACh activity, MT was examined in 6 pts before and after of rivastigmine (separate from main focus of study) not significant
	HC	12	-	73.1	5.4	-	-	-	-	MMSE >24							57.9	11.7									
Di Lorenzo et al. (2013)	AD	12	-	69.8	4.9	-	-	"new diagnosis of AD"	moderate	MMSE 21.08 (±3.9); CDR 1.21 (±1.1)	figure-8 shaped, high power Magstim 200, 9cm diameter, posterior-->anterior direction, held at optimum scalp position	D150 amplifer	MRI	-	L M1	FDI	45.9	2.1	50 uV, 5/10 trials	-	p<0.05	AD group had significantly lower RMT	-	-	-	-	-	none
	HC	12	-	71.7	4.4	-	-	-	-	-							49.9	1.1									
Di Lorenzo et al. (2016)	AD	54	48% F	67.9	0.8	8.7	4.4	-	-	MMSE 22.09 (±0.50); CDR 0.8 (±0.8)	figure-8 shaped, Magstim 200, monophasic, 70mm diameter	digitimer D360	MRI/CT, PET/CT	-	L M1	FDI	37.2	0.93	50 uV, 5/10 trials	ANOVA	p = 0.003	AD group had significantly lower RMT	-	-	-	-	-	none
	HC	24	50% F	66.2	1	9.4	4.2	1.11 (±0.36)	-	-							42.2	1.09									

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Article	Group	N	Gender Ratio (M/F) or %F	Age mean ± SD or [range]		Education mean ± SD		Disease Duration in yrs, (SD), [range]	Disease Stage	Neuropsych testing mean, (SD), [range]	Stimulation Parameters	EMG Status	Imaging	Neuronavigation Status	Stimulation Site	Muscle recorded	Resting Motor Threshold (RMT)		Definition of RMT (minimum uV, #trials)	Test for comparison	Significance	Conclusion [1]	Neuropsychological test used	Method of correlation	correlation value (r)	Significance of the relationship	Conclusion	Meds?
Di Lazzaro et al. (2008)	AD	12	-	69.3	7.3	9.1	4.3	2.67 (±1.09)	matched severity to VaD group	MMSE 22.7 (±2.7)	figure-8 shaped, high power Magstim 200	digitimer D360	MRI	-	R M1	FDI	49.6	8.5	~50 uV, 5/10 trials	ANOVA; Fisher's PLS	p<0.05	AD group had significantly lower RMT	-	-	-	-	-	none
	HC	12	-	73.1	5.4	-	-	-	-	MMSE ≥29							57.9	11.7										
Alagona et al. (2001)	AD	21	9/12	72 [median]	55-81 [range]	-	-	> 1 year	mild 5, moderate 7, severe 9	-	circular coil, Magstim 200, 9cm diameter, lateral to hemisphere	surface electrodes, Medelec Premier, gains - 100 uV, 5 mV/div, band pass - 30 Hz - 3kHz	CT, MRI	-	R M1, L M1	FDI	LH:36.76; RH: 36.95		20 uV, 3/5 trials	Mann-Whitney "for median values	p,0.001	AD group had significantly lower RMT	BIDS levels	linear regression	-	p < 0.01	"MEP threshold significantly lower in severe stage than mild one"	none
	HC	18	13/5	69 [median]	52-82 [range]	-	-	-	-	MMSE							LH=46% median, range:36-80%; RH=45%median, range:34-70%											
Alagona et al. (2004)	AD	20	7/13	72.2	7.53	-	-	-	probable AD	-	circular coil, Magstim 200, 9cm diameter, lateral to hemisphere	surface electrodes, Medelec Premier, gains - 100 uV, 5 mV/div, bandpass filter - 30 Hz - 3kHz	MRI	-		FDI	36	3.02	20 uV, 50% of 10-20 trials	ANOVA, post hoc test	p = 0.000001 (post hoc)	AD group had significantly lower RMT	-	-	-	-	-	none
	HC	20	8/12	68.55	7.96	-	-	-	-	-							49.1	4.21										
Nardone et al. (2006)	AD	13	-	69.6	6.6	14.2	2.8	2.68 (±1.29)	probable AD	MMSE 24.2 (±2.8), DRS 125.8 (±8.0)	figure-8 shaped, high power Magstim 200, diameter 90mm	D150 amplifier, surface electrodes	-	-	Dominant hemisphere	FDI	46.8	12.3	50 uV, 5/10 trials	ANOVA	P>0.05	AD group had lower RMT, but not significant	-	-	-	-	-	none
	HC	15	-	67.5	7.2	-	-	-	-	-							53.5	10.6										
Koch et al. (2011)	AD	10	5/5	72.5	6.1	-	-	6.1	moderate	MMSE 20.2	figure-8 shaped, monophasic Magstim 200 to determine hotspot; figure-8 shaped, Magstim SuperRapid to measure RMT, coil held 45 degrees from the midsagittal line with the handle pointing backwards	digitimer 360	MRI	-	Dominant hand	FDI	46.8	2.5	50 uV, 5/10 trials	ANOVA	p=0.034	AD group had significantly lower RMT	-	-	-	-	-	same experiment performed in two separate sessions after the administration of a sinlge dose of 100 mg of L Dopa and 25 mg benserazide or placebo, performed at least one week apart; LDOPA RMT also significant p=0.029
	HC	10	-	71.7	4.9	-	-	67.5 (±7.5)	-	-							50.3	3.2										
Benussi et al. (2018)	AD	63	50.8% F	71.7	7.8	-	-	-	mild, moderate	MMSE 20.4 (±6.2)	figure-8 shaped, Magstim Bistim2, diameter 70mm, monophasic	Biopac MP-150	-	-	LH M1	FDI	43.1	8.2	50 uV, 5/10 trials	ANOVA	p>0.05	not significantly different between groups	-	-	-	-	-	none
	HC	39	66.7% F	68.6	8.1	-	-	-	-	-							44.5	8										
Brem et al. (2013)	AD	16	-	69.89	5.64	16.52	3.93	-	mild, moderate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	AChEI and memantine
	HC	13	-	67.76	6.05	15.77	2.17	-	-	-																		yes
Alberici et al. (2008)	AD	8	62.5% F	74.5	7.3	4.8	0.4	2.5 (±0.6)	mild, moderate	MMSE 20.2 (±4.0)	circular coil, Magstim Bistim, diameter 90mm, centered at vertex tangential to scalp (anticlockwise current)	Ag/AgCl electrodes in belly/tendon montage, impedence < 10 kOhm, bandpass filter 1-2000 Hz, sampling rate 5-KHz	-	-	R M1, L M1	FDI	51.2	7.8	50 uV, 5/10 trials	ANOVA	-	not significantly different between groups	-	-	-	-	-	AD subjects: chronic AChEI-users
	HC	8	50% F	63.1	7.5	7.1	2.6	-	-	MMSE 27.9 (±1.2)							54.2	6.5										
Battaglia et al. (2007)	AD	10	40% F	70.1	7.4	-	-	1.2 (±0.67)	mild, moderate	MMSE 20.02 (±3.9); ADAS-Cog 24.4 (±8)	figure-8 shaped, high power Magstim 200, diameter 9cm, held laterally at 45 degrees to sagittal line	Ag/AgCl electrodes, bell/tendon montage, band pass filter- 3 kHz, sampling rate- 5 kHz, with visual oscilloscope & auditory feedback	MRI	-	L M1	APB	47.01	2.96	50 uV, 5/10 trials	ANOVA	p= 0.29	not significantly different between groups	-	-	-	-	-	none
	HC	10	40% F	68.4	6.1	-	-	-	-	MMSE 27.9 (±1.8); ADAS-Cog 8.8 (±3.5)							51.36	2.66										
	AD	15	-	75	5	11.05	4.03	4.60 (±1.84)	probable AD	MMSE 22.06 (±2.3)	high power Magstim 200, monophasic,	Digitimer D360 amplifier, bandpass filter-					39.5	6.2										meds originally reported, for a subgroup AChEI