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LTP-like cortical plasticity is disrupted in Alzheimer's disease patients independently from age of onset.

Francesco Di Lorenzo MD,^{1,2} Viviana Ponzio B.Sc.,¹ Sonia Bonni PhD,¹ Caterina Motta,^{1,2} Priscilla C. Negrão Serra MD², Marco Bozzali MD PhD,³ Carlo Caltagirone MD,^{1,2} Alessandro Martorana MD PhD,² Giacomo Koch MD PhD^{1,4}

1. Non Invasive Brain Stimulation Unit/Department of Behavioural and Clinical Neurology, Santa Lucia Foundation IRCCS, Via Ardeatina 354, 00179, Rome, Italy
2. Department of Systems Medicine, University of Rome Tor Vergata, Viale Oxford 81, 00133, Rome, Italy
3. Neuroimaging Laboratory, Santa Lucia Foundation, IRCCS, Rome, 00179, Italy.
4. Stroke Unit, Department of Neuroscience, Tor Vergata Policlinic, Viale Oxford 81, 00133, Rome, Italy

Correspondence to: Dr. Giacomo Koch, MD PhD, Non Invasive Brain Stimulation Unit, Laboratorio di Neurologia Clinica e Comportamentale, IRCCS Fondazione S. Lucia, Via Ardeatina, 306- 00179-Rome, Italy. Phone number +39 0651501181. E-mail address: g.koch@hsantalucia.it

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Abstract

Objective: Alzheimer's disease (AD) is considered an age-related disorder. However, it is unclear if AD induces the same pathological and neurophysiological modifications in synaptic functions independently from age of disease onset. We used transcranial magnetic stimulation tools to investigate the mechanisms of cortical plasticity and sensory-motor integration in AD patients with a wide range of disease onset.

Methods: We evaluated newly diagnosed sporadic AD (n=54) in comparison with healthy age-matched controls (HS n=24). Cortical plasticity mechanisms of long-term potentiation (LTP) or of long-term depression (LTD) were assessed using respectively intermittent (iTBS) or continuous theta burst stimulation (cTBS) protocols. Sensory-motor integration was evaluated by means of short afferent inhibition (SAI) protocol.

Results: AD patients show after iTBS an impairment of LTP-like cortical plasticity forming a paradoxical LTD in comparison to HS. LTD-like cortical plasticity is similar between AD and HS. LTP-like cortical plasticity is not associated with age, but AD patients presenting with more altered LTP-like cortical plasticity have more severe cognitive decline at 18 months. SAI is impaired in AD and shows a strong association with the individual age of subjects rather than with disease age of onset.

Interpretation: Cortical LTP disruption is a central mechanism of AD that is independent from age of onset. AD can be described primarily as a disorder of LTP-like cortical plasticity not influenced by physiological ageing and associated with a more severe cognitive decline.

Introduction

Albeit typically considered an age-related disorder, in the last years there has been a growing interest in the early detection of Alzheimer's disease (AD) with the development of new biomarkers and genetic techniques. This increasingly awareness about Early Onset Alzheimer's Disease (EOAD) is raising from demographic and social issues, since these patients start to complain their first cognitive symptoms when they still are a mainstay within the society, thus representing a huge burden to health and economic system. EOAD conventionally indicates patients with onset of AD before 65 years of age¹, while AD patients with a more common disease onset >65 years of age can be classified as Late Onset AD (LOAD)¹. Despite pathological studies seem to indicate that EOAD and LOAD share the same features and represent a continuum of the same pathological process, it is still debated whether EOAD and LOAD clinically manifest the same neuropsychological symptoms^{2, 3, 4, 5} or are characterized by the same imaging patterns^{6, 7, 8}. However, this cutoff point is considered arbitrary since it is rather due to sociological/demographic aspects and it has no specific biological significance.¹

In the recent years transcranial magnetic stimulation (TMS) has been employed to investigate key neurophysiologic and pathophysiologic aspects of AD patients in vivo^{9, 10, 11}. Several studies using TMS have claimed the presence of abnormalities in cortical reactivity, plasticity and connectivity in AD patients. One of the most consistent finding is a relative impairment of short-latency afferent inhibition (SAI), a protocol that measures sensory-motor integration that is partially mediated by central cholinergic transmission and it is commonly found altered in patients with AD¹². Nevertheless, SAI is known to be reduced by aging in healthy controls^{13, 14}, thereby questioning whether this neurophysiological marker may be specific for AD. Recently, abnormalities of cortical plasticity have been demonstrated in AD using repetitive TMS. These studies were based on the strong evidence obtained by electrophysiological recordings in AD animal models^{15, 16} showing that cortical plasticity is dampened by A β peptides and tau proteins; in particular these molecules are able to disrupt

hippocampal long-term potentiation (LTP), an electrophysiological correlate of learning and memory and to increase long term depression (LTD), which has been related to increased apoptosis^{15, 16}.

According to this background, we recently found, using protocols of theta burst stimulation (TBS), that LTP-like cortical plasticity is abolished or even pathologically reverted towards LTD in AD patients, while LTD is preserved or even enhanced^{11,17}. Here we used these TMS methods to compare neurophysiological markers of sensory-motor integration (assessed by SAI) and cortical plasticity (assessed by TBS) in AD patients with a wide range of disease onset, from early to late age of onset. We hypothesized that altered cortical plasticity should be a common feature of AD independently from age of onset, while the impairment of SAI would be more sensitive to the underlying mechanisms of aging^{13, 14}.

Methods

Subjects

Fifty-four consecutive patients (ranged 55-80 years, median 68.5) were recruited at the memory clinic of the University Hospital Tor Vergata, admitted for complaining memory symptoms. Patients fulfilled the clinical criteria of dementia as defined by the DSM-IV and probable or possible AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association¹⁸. Disease duration was calculated using standardized semi structured questions¹⁹. After the first visit to our Centre, all patients underwent for diagnostic purposes a complete clinical investigation in a period not superior to 60 days, including medical history, neurological examination, Mini-Mental State Examination (MMSE), a complete blood screening, neuropsychological assessment including the following cognitive domains: general cognitive efficiency: MMSE^{20,21}; verbal episodic long-term memory: Rey auditory verbal long term memory (15-Word List Immediate and 15-min Delayed recall)²²; visuo-spatial abilities and visuo-spatial episodic

long-term memory: Complex Rey's Figure (copy and 10-min Delayed recall)²³; executive functions: phonological word fluency²⁴; analogic reasoning: Raven's Colored Progressive Matrices²⁴. Patients underwent also a neuropsychiatric evaluation, magnetic resonance or CT imaging, PET/CT, and lumbar puncture for CSF analysis (**Table 1**). Exclusion criteria were the following: patients with isolated deficits, with clinically manifest acute stroke in the last 6 months showing a Hachinsky scale score >4, and a radiological evidence of ischemic lesions, A β 1-42 CSF values >600 pg/mL.

Neurophysiological examinations were performed at the Santa Lucia Foundation within 30 days from CSF sampling. In the 90 days prior to TMS evaluation, none of the patients were treated with drugs that could have modulated cerebral cortex excitability such as Acetylcholinesterase inhibitors (AChEI)¹⁰, antidepressants or any other neuroactive drugs (i.e. benzodiazepines, anti-epileptic drugs or neuroleptics). After the neurophysiological assessment all patients started treatment with rivastigmine patch (n=26) or donepezil (n=28) and were followed longitudinally with clinical assessments and MMSE testing at 6, 12 and 18 months. Twenty-four age, sex- and education-matched healthy subjects (HS) (ranged 58-73 years, median 67) were recruited as controls. All participants or their legal guardian provided written informed consent after receiving an extensive description of the study. The study was performed according to the Declaration of Helsinki. The ethics committee of the Santa Lucia Foundation IRCSS approved this protocol (Prot. CE/AG4/PROG.392-08).

CSF biomarkers analysis

The first 12 mL of CSF were collected in a polypropylene tube and directly transported to the local laboratory for centrifugation at 2000 \times g at +4°C for 10 minutes. The supernatant was pipetted off, gently stirred and mixed to avoid potential gradient effects, and aliquoted in 1 mL portions in polypropylene tubes that were stored at -80°C pending biochemical analyses, without being thawed and re-frozen. CSF t-tau and p-tau phosphorylated at Thr181 concentrations were determined using a

sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Gent, Belgium). A β 1-42 levels were determined using a sandwich ELISA (Innotest $\text{\textcircled{R}}$ β - amyloid (1–42), Innogenetics, Gent, Belgium), specifically constructed to measure A β -amyloid containing both the first and 42nd amino-acid, as previously described²⁵.

Transcranial magnetic stimulation

All patients and healthy controls underwent continuous TBS (cTBS), intermittent TBS (iTBS) and SAI protocols in three different sessions, with at least a three day interval between each session. The order of the sessions was pseudo-randomized across patients and healthy controls. Motor evoked potentials (MEP) were recorded from the right first dorsal interosseous muscle using 9 mm diameter, Ag–AgCl surface cup electrodes. Responses were amplified with a Digitimer D360 amplifier (Digitimer Ltd, UK) and filtered (20 Hz-2 kHz), then recorded by computer using SIGNAL software with a sampling rate of 5 kHz per channel (Cambridge Electronic Devices, UK). A monophasic Magstim 200 device (Magstim Co, UK) was used to define the motor hot spot and to assess MEP size using standard 70 mm figure-of-eight shaped coil. The motor hot-spot was defined as the location where monophasic TMS pulses consistently produced the largest MEP size at 120% of resting motor threshold (RMT) in the target muscle. RMT was defined as the minimum stimulus intensity that produced motor evoked response of 50 μ V in at least 5 of 10 trials at rest²⁶.

A second coil was connected to a biphasic Super Rapid Magstim stimulator (Magstim Co, UK) to deliver TBS. The active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a liminal motor evoked response (about 200 μ V in 50% of trials) during isometric contraction of the tested muscle at about 10% of maximum force as measured through a manual transducer.

In the cTBS protocol bursts at 80% AMT were repeated at 5 Hz (i.e. every 200 milliseconds), while each burst consisted of three stimuli repeating at 50 Hz, for 40 seconds (600 pulses). In the iTBS

protocol, a 2 second train of TBS was repeated 20 times, every 10 seconds for a total of 190 seconds (600 pulses)²⁷. The iTBS and cTBS protocols were tested after a period of relaxation of the target muscle. The change in corticospinal excitability produced by each intervention was assessed by measuring the amplitude of the MEP response to a standard test pulse that remained constant throughout the experiment. In each subject the intensity of the test pulse was individually adjusted at the start of the experiment to produce a stable MEP of 1 mV with the subject at rest. Twenty MEPs were collected and averaged at baseline. Then, over the same hot-spot, twenty MEPs were recorded at 1-5, 6-10, 11-15, 16-20 and 21-25 minutes after TBS and averaged. The inter-trial interval was set at 5 seconds ($\pm 10\%$) for individual MEPs within each block.

SAI was studied using the technique that has been recently described^{28, 29}. Conditioning stimuli were single pulses (200 μ s) of electrical stimulation applied through bipolar electrodes to the right median nerve at the wrist (cathode proximal). The intensity of the conditioning stimulus was set at just over motor threshold for evoking a visible twitch of the thenar muscles. The intensity of the test cortical magnetic stimulus was adjusted to evoke a MEP in the relaxed right first dorsal interosseous with amplitude of approximately 1 mV peak to peak. For N20 recordings cup electrodes were placed over the centroparietal contralateral position keeping the Fz as the reference (International 10–20 system). Electrical median nerve stimulation was applied at the right wrist at 2 Hz. The latency of the N20 component of somatosensory evoked potential was determined by averaging 200 trials. The conditioning stimuli to the peripheral nerve preceded the magnetic test stimulus by different interstimulus intervals (ISIs), ranging from -4 to +8 milliseconds from the N20 in steps of 4 milliseconds³⁰. Ten paired stimuli were delivered at each ISI. The subject was given audiovisual feedback at high gain to assist in maintaining complete relaxation. The inter-trial interval was set at 5 seconds ($\pm 10\%$), for a total duration of approximately five minutes. Measurements were made on each individual trial. The mean peak-to peak amplitude of the conditioned motor evoked potential at each

ISI was expressed as a percentage of the mean peak-to-peak amplitude size of the unconditioned test pulse in that block.

Data analysis

Data were analyzed using SPSS for Windows version 11.0. For TMS experiments, two-way repeated measure ANOVAs were performed on MEP amplitude expressed as percentage of change in comparison to baseline for each TBS protocol (cTBS and iTBS) with TIME (1–5, 6–10, 11–15, 16–20, and 21–25 min after TBS) as within subjects factors and GROUP (AD and HS) as between subjects factor. For SAI the electrophysiological parameters of AD patients were compared by means of repeated measures ANOVA with ISI (-4, 0, +4 and +8 milliseconds plus the latency of the N20) as within subject factors and GROUP (AD and HS) as between subjects factor. The Greenhouse-Geisser correction was used for non-spherical data. Mauchley's test examined for sphericity. When a significant main effect was reached, paired t-tests with Bonferroni correction were employed to characterize the different effects of the specific ISIs. Pearson's r coefficient was first used in univariate correlations in order to explore any influence the age could have on the individual amount of mean change across all time intervals induced by the iTBS and cTBS protocols and by SAI protocol at ISI=+4 in all subjects (AD patients and HS). In a second step, a multiple linear regression model was constructed for each protocol (iTBS, cTBS, SAI) to better characterize the relationship between each neurophysiological measure with age and disease as covariates in all subjects (AD patients and HS). We also performed Spearman only in AD patients correlation analyses between cognitive decline (delta score with baseline evaluation) and iTBS and cTBS induced cortical plasticity (individual mean value) and SAI protocol at ISI=+4. Furthermore, a multiple linear regression analysis was constructed in AD patients for each neurophysiological parameter (iTBS, cTBS, SAI) to determine their association with age, cognitive decline and disease duration. Correlation analyses were corrected for multiple

comparisons. Coefficients with standard error (SE), and 95% confidence interval (CI) were provided. A p value of < 0.05 was considered statistically significant.

Results

Transcranial magnetic stimulation

The TMS procedures were well tolerated in all subjects. RMT to TMS (mean \pm standard deviation (SD)) was lower in AD patients in comparison with HS (AD: $37.2\pm 0.93\%$; HS: $42.2\pm 1.09\%$; $p=0.003$). Baseline mean MEP amplitude did not differ between AD patients and HS across all protocols (AD: 1.12 ± 0.43 mV; HS: 1.15 ± 0.34 mV).

For the iTBS protocol, AD patients showed an altered LTP-like cortical plasticity, with a reversal of LTP-like cortical plasticity towards LTD in comparison with HS: there was an effect for the GROUP ($F(1,76)=63.72$, $p=0.000001$) and for the TIME ($F(4,304)=2.63$, $p=0.034$) main factors; the interaction GROUP x TIME was also significant ($F(4,304)=6.63$, $p=0.00004$). Post-hoc analysis with Bonferroni correction showed that AD patients differed from HS at 10, 15, 20 and 25 minutes time point (all $p<0.001$) (**Figure 1**). For the cTBS protocol the repeated measure ANOVA performed on the percentage changes of the mean MEP amplitude did not show any effect for the GROUP ($F(1,64)=0.12$, $p=0.96$), for the TIME main factor ($F(4,256)=0.97$, $p=0.42$) and for the GROUP x TIME interaction ($F(4,256)=0.66$, $p=0.61$) (**Figure 2**). The ANOVA analysis performed on SAI measurements showed an effect for the GROUP ($F(1,75)=5.29$, $p=0.02$) and ISI main factor ($F(3,225)=46.58$, $p=0.00001$), but not a GROUP x ISI interaction ($F(3,225)=0.32$, $p=0.80$) (**Figure 3**). Correlations analyses performed to explore any influence the age could have on the neurophysiological parameters did not show any correlation for iTBS (**Figure 4A**) and cTBS (**Figure 4B**) with age; on the other hand, we found that age correlated positively with the impairment of SAI ($r= 0.53$; $p=0.001$) (**Figure 4C**).

These results were confirmed by multiple linear regression analyses showing a strong association between iTBS induced cortical plasticity and diagnosis of AD, but no association with age. We did not find any association for cTBS induced cortical plasticity with any of the covariates. On the other hand, we found that SAI values were strongly associated with age and weakly with diagnosis of AD (**Table 2**).

Clinical follow-up

MMSE scores performed at follow up evaluations were 21.43 ± 0.67 (mean \pm SD) at 6 months, 20.14 ± 0.85 at 12 months and 18.21 ± 0.91 at 18 months. AD patients underwent a substantial cognitive decline as confirmed by ANOVA showing an effect for the TIME main factor ($F(3,156)=16.953$, $p<0.001$). Post-hoc analysis with Bonferroni correction showed that MMSE scores differed from baseline at 18 months follow up evaluation ($p<0.001$). Correlation analyses between cognitive decline (computed as delta score with baseline evaluation) and neurophysiological parameters (iTBS, cTBS and SAI) showed that AD patients presenting with more altered iTBS induced cortical plasticity had more severe cognitive decline at 18 months ($r=0.30$; $p=0.020$) (**Figure 5A**). We did not find any correlations for cTBS (**Figure 5B**) and SAI (**Figure 5C**) values. These results were further confirmed by multiple linear regression analyses showing a significant association between iTBS induced cortical plasticity and cognitive decline, at equal values of age and disease duration. We did not find any association for cTBS induced cortical plasticity. On the contrary, SAI values were not associated with cognitive decline but only with age (**Table 3**).

Discussion

We provide novel evidence that AD patients constantly tend to form LTD instead of LTP independently from age of disease onset. Notably, more altered LTP-like cortical plasticity is associated in AD patients with more severe cognitive decline at 18 months follow-up. On the other hand, SAI sensibly declines with age in both AD patients and healthy controls and it is not associated with cognitive decline at 18 months follow-up in AD patients.

Taken together, these results provide a compelling proof that in AD patients the LTP-like cortical plasticity machinery is already deeply dampened even when the disease occurs earlier, while sensory-motor integration is relatively spared. On the other hand, when the disease occurs later there is a clear impairment of both LTP-like cortical plasticity and SAI signaling. Thereby, we propose that LTP-like cortical plasticity is a core neurophysiological marker of AD-related dysfunction, clearly differentiating AD patients from healthy individuals independently from age of disease onset.

The current findings are supported by recent works consistently demonstrating that AD patients are characterized by abnormalities of LTP-like cortical plasticity^{11,12,31,32}. Notably these results are in most cases superimposable to experimental electrophysiological recordings obtained from animal models of AD^{15, 16, 33} revealing that both tau oligomers and amyloid peptides, the neuropathological hallmarks of AD pathology, are able to disrupt the processes occurring for a stable synaptic efficacy^{34, 35}. These pathological mechanisms induce on one hand a weakened hippocampal and cortical LTP and on the other a more robust LTD³⁶, a process related to apoptosis and degeneration. Interestingly, molecular studies showed that beta and tau pathology trigger a structural synaptic remodeling by forcing pro-apoptotic cell pathways, inducing a burdened effective synaptic activity³⁷. The progressive reduction of synaptic connections caused by the shrinkage of dendritic spines can be recorded with electrophysiological tools *in vitro* as imbalances of the physiological forms of long-term modifications

between networks of neurons establishing a high-order functional net³⁸, driving a marked propensity to form a more pronounced LTD plasticity.

At this regard, we recently found that AD patients with more pathological CSF tau levels are characterized by a stronger tendency to form LTD and that this neurophysiological biomarker is related to a more aggressive clinical course, implying that an altered cortical plasticity, eventually caused by tau pathology, is strictly linked to the underlying clinical progression of AD³⁹. Together with the current findings, these data indicate that, although tested in the motor cortex, rTMS can be considered a reliable tool to examine cortical plasticity in AD patients in analogy with hippocampal plasticity assessed in animal models of AD.

We did not find any correlation between SAI values and clinical worsening; on the contrary, LTP impairment correlates positively with clinical worsening overall in AD patients, strengthening the hypothesis of LTP disruption as key neurophysiological biomarker for both the pathogenesis and clinical progression of Alzheimer's disease, while SAI seems to reflect the physiological processes of ageing. Our findings are strengthened by several clinical and experimental evidence tracking cholinergic modifications during both physiological^{40, 41} and pathological aging processes^{42, 43, 44}. Basal forebrain cholinergic complex, critical for cognitive functions in humans by sprouting synaptic contacts in high-order cortical networks, is characterized by a selective neuronal vulnerability and during ageing is easily susceptible to undergo degenerative changes, resulting in cholinergic hypofunction⁴⁵.

SAI efficacy has been shown to be linked to cholinergic transmission¹⁰ and since it is selectively altered in AD patients⁹, it has been interpreted as measure of central cholinergic dysfunction, historical neurochemical marker of AD. Intriguingly, recent works showed a specific age-dependent alteration in the cortical circuits mediating SAI in the motor cortex of healthy subjects^{13, 14}. These results led us to conclude that the main electrophysiological marker of AD is the deep and early impairment of cortical plasticity machinery, whereas central cholinergic dysfunction could be secondary to a process in which

the physiological aging process takes part, and is likely accelerated by concomitant neural degeneration process.

The selective weakening of cortical plasticity mechanisms showed by AD patients, differently from SAI, gives new interesting insights also for a therapeutic approach: so far, most of the attention has been driven onto improving of cholinergic transmission, and actually AChEI represent the only pharmacological class approved for treatment of AD symptoms, although its scarce and short-lasting effects. On the other hand, the data presented here highlight the specificity of LTP impairment as marker of pathophysiological dysfunction in AD and, as such, it should be taken in account also for the adoption of new pharmacological strategies considering AD as a disorder of synaptic plasticity and related transmitters system. This view could promote novel drugs able to influence positively synaptic plasticity, such as dopamine, a strong neuromodulator of neuroplasticity in both healthy subjects⁴⁶ and AD patients¹⁷.

In conclusion, our data show that LTP mechanisms are altered in AD patients independently from age of disease onset. LTP impairment is AD-dependent, and could be considered as a neurophysiological marker of disease, while the SAI dysfunction is age-dependent thus representing more likely a marker of the interaction between physiological and pathological ageing⁴⁷.

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Authors' contribution: FDL CC AM GK conceived and designed the study; FDL VP SB PNS CM acquire and analyze data; FDL VP MB AM GK drafted manuscript and figures.

Conflicts of Interest: Nothing to report.

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Figure Legends

Legend to Figure 1. After effects of iTBS protocol on MEP amplitude in AD and HS. * indicate $p < 0.05$. Error bars indicate SEM.

Legend to Figure 2. After effects of cTBS protocol on MEP amplitude in AD and HS. Error bars indicate SEM.

Legend to Figure 3. Changes in MEP amplitude for the SAI protocol in AD and HS. * indicate $p < 0.05$. Error bars indicate SEM.

Legend to Figure 4. Pearson's r correlation matrices between the age and individual amount of mean change in MEP amplitude induced by iTBS (A) and cTBS (B), and SAI (C) protocol in healthy subjects and AD patients.

Legend to Figure 5. Spearman correlation matrices in all AD patients between the individual amount of mean change in MEP amplitude induced by iTBS (A), cTBS (B) and SAI (C) protocol and the cognitive progression expressed in delta MMSE scores at 18 months follow up.

Table 1. Demographic and clinical characteristics of AD patients and Healthy Subjects.

	AD (n=54)	HS (n=24)	<i>p</i>	<i>95% C.I.</i>
Age at baseline, y (mean±SD)_a	67.9±0.8	66.2±1.0	0.19	-4.74 – 0.97
Female (%)_b	48	50	1.00	0.35 – 2.43
Formal education, y (mean±SD)_a	8.7±4.4	9.4±4.2	0.79	-1.91 – 2.48
Diabetes (%)_b	17	20	0.75	0.22 – 2.57
Hypertension (%)_b	39	33	0.45	0.55 – 4.36
Hpercolesterolemia (%)_b	18	20	0.75	0.22 – 2.57
Head injury (%)_b	0	0	1.00	n.a.
CSF Beta 1-42 pg/mL(mean±SD)	368.1±29	–	–	–
CSF total tau pg/mL(mean±SD)	703.2±50	–	–	–
CSF p-tau pg/mL(mean±SD)	87.4±6	–	–	–
CDR	0.8±0.8	–	–	–
ADL	5.6±0.5	–	–	–
IADL	7.5±0.5	–	–	–
MMSE baseline	22.09±0.5	–	–	–
Disease duration, m(mean±SD)_a	13.4±4.4	–	–	–
E4 (E3/E4 + E4/E4) (%)_b	41	–	–	–

Abbreviations: n=numbers; y=years; m=months; C.I. = confidence interval; CSF= Cerebrospinal Fluid; CDR=Clinical Dementia Rating; ADL=Activities of Daily Living; IADL=Instrumental Activities of Daily Living n.a.= not applicable; a=Student t-test; b=Fisher’s exact test. C.I. for continuous variables were calculated on differences between means. C.I for dichotomous variables were calculated on odds ratios.

Table 2. Multivariable linear regression: relationship between TMS parameters, age and diagnosis of Alzheimer’s disease across all subjects.

iTBS	Coefficient	SE	p value	CI
HS/AD	-51.49	6.16	<0.001*	-63.76 – -39.22
Age	0.32	0.49	0.51	-0.64 – 1.28
cTBS				
HS/AD	-1.34	5.97	0.82	-13.24 – 10.56
Age	-0.51	0.47	0.28	-1.44 – 0.42
SAI				
HS/AD	10.83	5.31	0.04*	0.23 – 21.42
Age	2.17	0.42	<0.001*	1.32 – 3.02

Abbreviations: iTBS= intermittent Theta Burst Stimulation; SE= standard error; CI= confidence interval; cTBS= continuous Theta Burst Stimulation; HS= healthy subjects; AD= Alzheimer’s disease; SAI= short-latency afferent inhibition.

Table 3. Multivariable linear regression: relationship between TMS parameters, age, disease duration and cognitive decline in AD patients.

iTBS	Coefficient	SE	p value	CI
Age	0.62	0.52	0.23	-0.41 – 1.66
Delta MMSE	1.46	0.65	0.03*	0.15 – 2.78
disease duration	-0.79	0.71	0.27	-2.22 – 0.64
cTBS				
Age	-1.01	0.54	0.08	-2.10 – 0.08
Delta MMSE	0.15	0.69	0.83	-1.24 – 1.54
disease duration	-0.42	0.75	0.58	-1.93 – 1.09
SAI				
Age	2.09	0.56	0.001*	0.96 – 3.22
Delta MMSE	0.34	0.70	0.63	-1.07 – 1.75
disease duration	0.09	0.75	0.89	-1.42 – 1.61

Abbreviations: LTP= long term potentiation; SE= standard error; CI= confidence interval; MMSE= Mini Mental State Examination; SAI= short-latency afferent inhibition.