Long-Term Potentiation–Like Cortical Plasticity Is Disrupted in Alzheimer's Disease Patients Independently from Age of Onset

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Objective: Alzheimer's disease (AD) is considered an age-related disorder. However, it is unclear whether 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 aging and associated with a more-severe cognitive decline.

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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 increasing awareness about early onset Alzheimer's disease (EOAD) is being raised from demographic and social issues, given that these patients start to complain of their first cognitive symptoms when they still are a mainstay within the society, thus representing a huge burden to health and the economic system. EOAD conventionally indicates patients with onset of AD before 65 years of age,[1] whereas 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[2–5]

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or are characterized by the same imaging patterns.[6–8] However, this cut-off point is considered arbitrary given that it is rather attributed to sociological/demographic aspects and it has no specific biological significance.[1]

In recent years, transcranial magnetic stimulation (TMS) has been used to investigate key neurophysiological and pathophysiological aspects of AD patients in vivo.[9–11] Several studies using TMS have claimed the presence of abnormalities in cortical reactivity, plasticity, and connectivity in AD patients. One of the most consistent findings 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 to be altered in patients with AD.[12] 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 amyloid-beta (Aβ) 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 toward LTD in AD patients, whereas 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, starting from early until late age. We hypothesized that altered cortical plasticity should be a common feature of AD independently from age of onset, whereas the impairment of SAI would be more sensitive to the underlying mechanisms of aging.[13,14]

Subjects and Methods

Subjects

Fifty-four consecutive patients (range, 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 Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition 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 Association.[18] Disease duration was calculated using standardized semistructured questions.[19] After the first visit to our center, 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, and 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]; visuospatial abilities and visuospatial episodic long-term memory: Complex Rey's Figure (copy and 10-min Delayed recall)[23]; and executive functions: phonological word fluency[24]; analogic reasoning: Raven's Colored Progressive Matrices.[24] Patients underwent also a neuropsychiatric evaluation, magnetic resonance or computed tomography (CT) imaging, positron emission tomography/CT, and lumbar puncture for cerebrospinal fluid (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, Aβ1-42 CSF values >600pg/mL.

Neurophysiological examinations were performed at the Santa Lucia Foundation within 30 days from CSF sampling. In the 90 days preceding TMS evaluation, none of the patients were treated with drugs that could have modulated cerebral cortex excitability such as acetylcholinesterase inhibitors (AChEIs),[10] antidepressants or any other neuroactive drugs (ie, benzodiazepines, antiepileptic 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; range, 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 12mL of CSF were collected in a polypropylene tube and directly transported to the local laboratory for centrifugation at 2,000*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 refrozen. CSF t-tau and p-tau phosphorylated at Thr181 concentrations were determined using a sandwich enzyme-linked immunosorbent assay (ELISA; Innotest hTAU-Ag; Innogenetics, Gent, Belgium). Aβ1-42 levels were determined using a sandwich ELISA (Innotest ß-amyloid [1–42]; Innogenetics), specifically constructed to measure Aβ-amyloid containing both the first and 42nd amino acid, as previously described.[25]

TABLE 1. Demographic and Clinical Characteristics of AD Patients and Healthy Subjects

	AD (n = 54)	HS (n = 24)	*p*	95% CI
Age at baseline, y (mean ± SD)[a]	67.9 ± 0.8	66.2 ± 1.0	0.19	–4.74 to 0.97
Female (%)[b]	48	50	1.00	0.35 to 2.43
Formal education, yr (mean ± SD)[a]	8.7 ± 4.4	9.4 ± 4.2	0.79	–1.91 to 2.48
Diabetes (%)[b]	17	20	0.75	0.22 to 2.57
Hypertension (%)[b]	39	33	0.45	0.55 to 4.36
Hpercolesterolemia (%)[b]	18	20	0.75	0.22 to 2.57
Head injury (%)[b]	0	0	1.00	n.a.
CSF beta 1-42 pg/mL (mean ± SD)	368.1 ± 29.0	—	—	—
CSF total tau pg/mL (mean ± SD)	703.2 ± 50.0	—	—	—
CSF p-tau pg/mL (mean ± SD)	87.4 ± 6.0	—	—	—
CDR	0.8 ± 0.8	—	—	—
ADL	5.6 ± 0.5	—	—	—
IADL	7.5 ± 0.5	—	—	—
MMSE baseline	22.09 ± 0.50	—	—	—
Disease duration, M (mean ± SD)[a]	13.4 ± 4.4	—	—	—
E4 (E3/E4 + E4/E4) (%)[b]	41	—	—	—

CIs for continuous variables were calculated on differences between means. CIs for dichotomous variables were calculated on odds ratios.
[a]Student *t* test.
[b]Fisher's exact test.
n = numbers; yr = years; m = months; CI = confidence interval; CSF = cerebrospinal fluid; CDR = Clinical Dementia Rating; ADL = Activities of Daily Living; IADL = Instrumental Activities of Daily Living; MMSE = Mini–Mental State Examination; n.a. = not applicable.

TMS

All patients and healthy controls underwent continuous TBS (cTBS), intermittent TBS (iTBS), and SAI protocols in three different sessions, with at least a 3-day interval between each session. The order of the sessions was pseudorandomized across patients and healthy controls. Motor evoked potentials (MEPs) 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, Welwyn Garden City, UK) and filtered (20Hz–2kHz), then recorded by computer using SIGNAL software with a sampling rate of 5kHz per channel (Cambridge Electronic Devices, Cambridge, UK). A monophasic Magstim 200 device (Magstim Co, Whitland, UK) was used to define the motor hotspot and to assess MEP size using a standard 70-mm figure-of-eight–shaped coil. The motor hotspot 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 50μV in at least 5 of 10 trials at rest.[26]

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

In the cTBS protocol, bursts at 80% AMT were repeated at 5Hz (ie, every 200 milliseconds), while each burst consisted of three stimuli repeating at 50Hz, 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 1mV with the subject at rest. Twenty MEPs were collected and averaged at baseline. Then, over the same hotspot, 20 MEPs were recorded at 1 to 5, 6 to 10, 11 to 15, 16 to 20, and 21 to 25 minutes after TBS and averaged. The intertrial interval was set at 5 seconds (±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-μs) of electrical stimulation applied through bipolar electrodes to the right median nerve at the wrist (cathode proximal). 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 an MEP in the relaxed right first dorsal interosseous with amplitude of approximately 1mV 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 2Hz. 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 milliseconds.[30] Ten paired stimuli were delivered at each ISI. The subject was given audiovisual feedback at high gain to assist in maintaining complete relaxation. The intertrial interval was set at 5 seconds (±10%), for a total duration of approximately 5 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.

Statistical Analysis

Data were analyzed using SPSS for Windows (version 11.0; SPSS, Inc., Chicago, IL). For TMS experiments, two-way repeated-measures analyses of variance (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 minutes after TBS) as within-subjects factors and Group (AD and HS) as the 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 the between-subjects factor. The Greenhouse-Geisser correction was used for nonspherical data. Mauchley's test examined for sphericity. When a significant main effect was reached, paired t tests with Bonferroni's correction were used to characterize the different effects of the specific Time points or ISIs. Pearson's r coefficient was used in univariate correlations in order to explore any influence age could have in all subjects (AD patients and HS) on the iTBS and cTBS protocols (calculated on the individual amount of mean change across all time intervals) and on the SAI protocol (calculated as the individual amount of change at ISI = +4 milliseconds). In a second step, a multiple linear regression model was constructed for each protocol (iTBS, cTBS, and SAI) in all subjects (AD patients and HS) to better characterize the relationship between each neurophysiological measure with the covariates, age and diagnosis of AD. We also performed, only in AD patients, Spearman's correlation analyses between cognitive decline (delta score with baseline evaluation) and iTBS/cTBS-induced cortical plasticity (individual mean value) and SAI protocol (ISI = +4 milliseconds). Furthermore, a multiple linear regression analysis was constructed in AD patients for each neurophysiological parameter (iTBS, cTBS, and SAI) to determine their association with the covariates, 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

TMS

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

For the iTBS protocol, AD patients showed an altered LTP-like cortical plasticity, with a reversal of LTP-like cortical plasticity toward LTD in comparison to 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 × Time was also significant ($F_{(4,304)} = 6.63$; $p = 0.00004$). Post-hoc analysis with Bonferroni's correction showed that AD patients differed from HS at 10, 15, 20, and 25 minutes time points (all $p < 0.001$; Fig 1). For the cTBS protocol, the repeated-measures ANOVA performed on the mean MEP amplitude percentage change did not show any effect for the Group ($F_{(1,64)} = 0.12$; $p = 0.96$), Time main factor ($F_{(4,256)} = 0.97$; $p = 0.42$), and Group × Time interaction ($F_{(4,256)} = 0.66$; $p = 0.61$; Fig 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 factors ($F_{(3,225)} = 46.58$; $p = 0.00001$), but not a Group × ISI interaction ($F_{(3,225)} = 0.32$; $p = 0.80$; Fig 3).

Correlations analyses were performed first to explore any influence age could have on the neurophysiological parameters across all subjects. There was no correlation for iTBS (Fig 4A) and cTBS (Fig 4B) protocols with age; on the other hand, we found that age correlated positively with impairment of SAI ($r = 0.53$; $p = 0.001$; Fig 4C).

These results were then 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

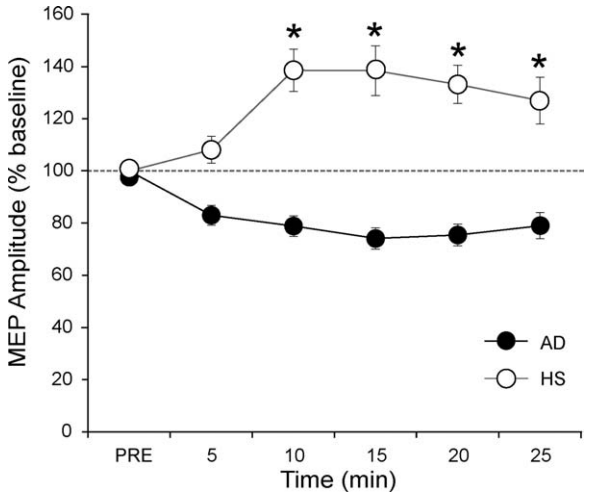

FIGURE 1: After effects of iTBS protocol on MEP amplitude in AD and HS. *$p < 0.05$. Error bars indicate standard error of the mean. AD = Alzheimer's disease; HS = healthy subjects; iTBS = intermittent theta burst stimulation; MEP = motor evoked potential.

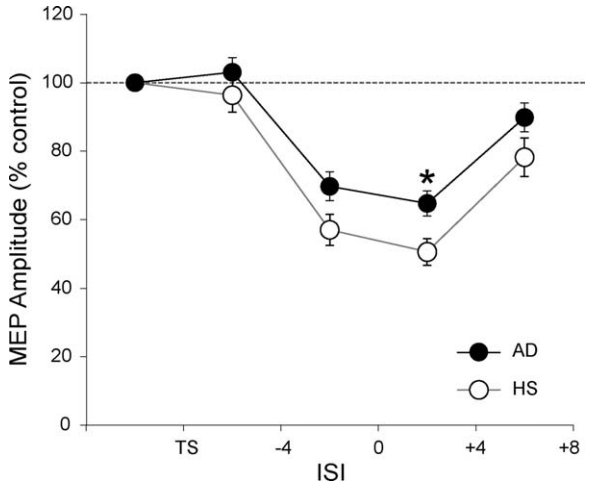

FIGURE 3: Changes in MEP amplitude for the SAI protocol in AD and HS. *$p < 0.05$. Error bars indicate standard error of the mean. AD = Alzheimer's disease; HS = healthy subjects; ISI = interstimulus interval; MEP = motor evoked potential; SAI = short-latency afferent inhibition; TS = Test Stimulus.

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 were 21.43 ± 0.67 (mean ± SD) at 6 months, 20.14 ± 0.85 at 12 months, and 18.21 ± 0.91 at 18 months follow-up evaluations. 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's 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 ($r = 0.30$; $p = 0.020$; Fig 5A). We did not find any correlations for cTBS (Fig 5B) and SAI (Fig 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).

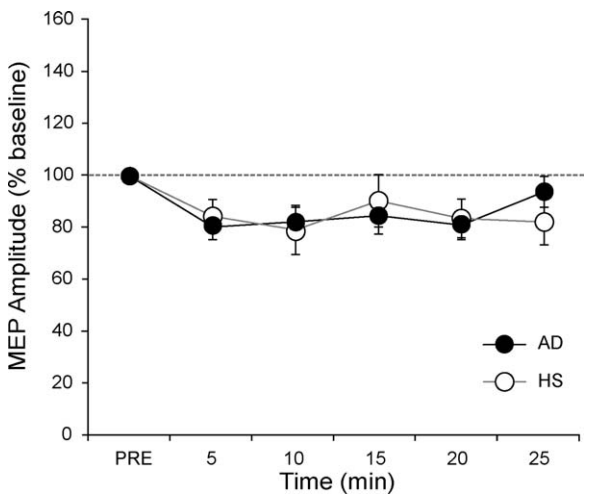

FIGURE 2: After effects of cTBS protocol on MEP amplitude in AD and HS. Error bars indicate standard error of the mean. AD = Alzheimer's disease; cTBS = continuous theta burst stimulation; HS = healthy subjects; MEP = motor evoked potential.

Discussion

We provide novel evidence that AD patients constantly tend to form a paradoxical 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, whereas sensory-motor integration is relatively spared. On the other hand, when the disease

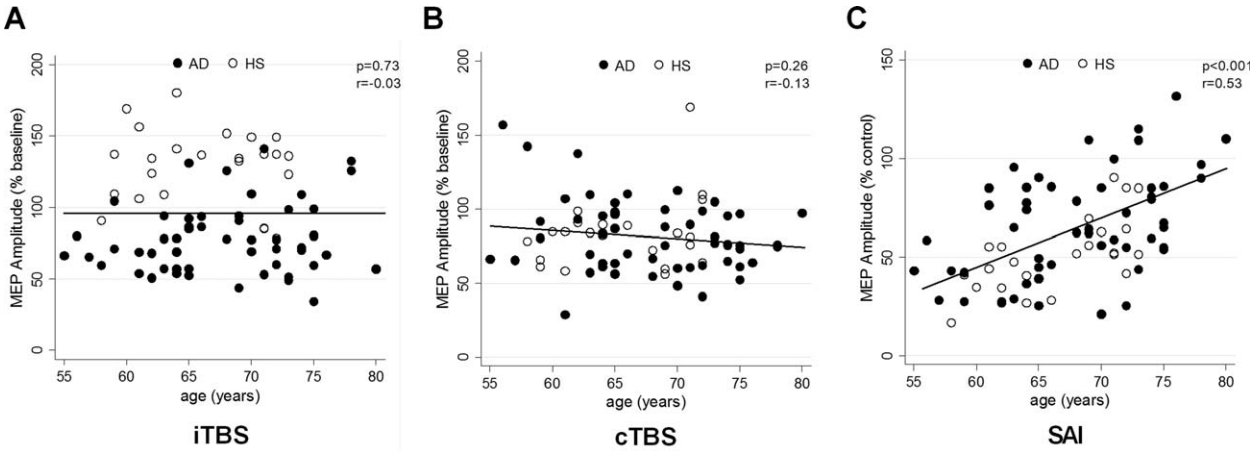

FIGURE 4: Correlation matrices between age (x-axis) and the individual amount of MEP amplitude change (y-axis) induced by iTBS (A) cTBS (B), and SAI (C) protocol in healthy subjects and AD patients. AD = Alzheimer's disease; cTBS = continuous theta burst stimulation; HS = healthy subjects; iTBS = intermittent theta burst stimulation; MEP = motor evoked potential; SAI = short-latency afferent inhibition.

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 LTD,[36] a process related to apoptosis and degeneration. Interestingly, molecular studies showed that beta and tau pathology trigger a structural synaptic remodeling by forcing proapoptotic cell pathways, inducing a burdened effective synaptic activity.[37] 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 net,[38] driving a marked propensity to form a more pronounced LTD plasticity.

In 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,

TABLE 2. Multivariable Linear Regression: Relationship Between TMS Parameters, Age, and Diagnosis of Alzheimer's Disease Across All Subjects

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

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.

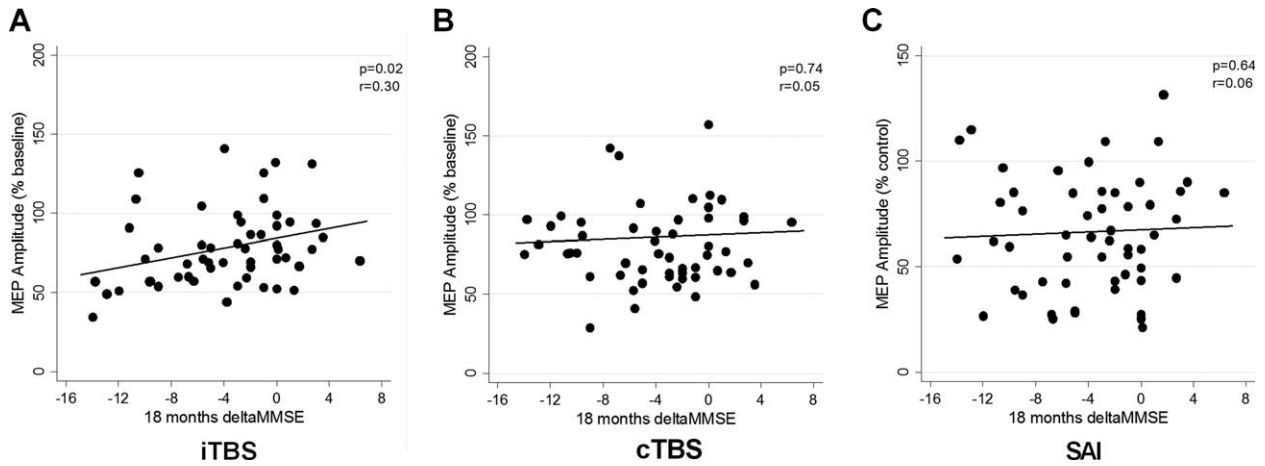

FIGURE 5: Correlation matrices between the cognitive progression expressed in delta MMSE scores at 18 months follow up (x axis) and the individual amount of MEP amplitude change (y axis) induced by iTBS (A) cTBS (B), and SAI (C) protocol in AD patients. cTBS = continuous theta burst stimulation; iTBS = intermittent theta burst stimulation; MEP = motor evoked potential; MMSE = Mini–Mental State Examination; SAI = short-latency afferent inhibition.

eventually caused by tau pathology, is strictly linked to the underlying clinical progression of AD.[39] Together with the current findings, these data indicate that, although tested in the motor cortex, repetitive TMS (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 in AD patients, strengthening the hypothesis of LTP disruption as a key neurophysiological biomarker for both the pathogenesis and clinical progression of AD, whereas SAI seems to reflect the physiological processes of aging. Our findings are strengthened by several clinical and experimental evidence tracking cholinergic modifications during both physiological[40,41] and pathological aging processes.[42–44] 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 aging is easily susceptible to undergo degenerative changes, resulting in cholinergic hypofunction.[45]

SAI efficacy has been shown to be linked to cholinergic transmission,[10] and given that it is selectively altered in AD patients,[9] it has been interpreted as a

TABLE 3. Multivariable Linear Regression: Relationship Between TMS Parameters, Age, Disease Duration, and Cognitive Decline in AD patients

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

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

measure of central cholinergic dysfunction, a 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 a 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, AChEIs represent the only pharmacological class approved for treatment of AD symptoms, although it has scarce and short-lasting effects. On the other hand, the data presented here highlight the specificity of LTP impairment as a 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 a related transmitter system. This view could promote novel drugs able to influence positively synaptic plasticity, such as dopamine, a strong neuromodulator of neuroplasticity in both healthy subjects[46] and AD patients.[17]

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, whereas SAI dysfunction is age dependent, thus representing more likely a marker of the interaction between physiological and pathological aging.[47]

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Author Contributions

F.D.L., C.C., A.M., and G.K. conceived and designed the study; F.D.L., V.P., S.B., P.N.S., and C.M. acquired and analyzed data; and F.D.L., V.P., M.B., A.M., and G.K. drafted the manuscript and figures.

Potential Conflicts of Interest

Nothing to report.

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