Brain Stimulation 13 (2020) 1175–1182

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

LTP-like cortical plasticity predicts conversion to dementia in patients with memory impairment

Francesco Di Lorenzo [a], Caterina Motta [a, c], Elias Paolo Casula [a], Sonia Bonnì [a], Martina Assogna [a], Carlo Caltagirone [a], Alessandro Martorana [a, c], Giacomo Koch [a, b, *]

[a] Non Invasive Brain Stimulation Unit/Department of Behavioral and Clinical Neurology, Santa Lucia Foundation IRCCS, Rome, Italy
[b] Section of Human Physiology, ECampus University, Novedrate, Italy
[c] Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy

ARTICLE INFO

Article history:
Received 30 March 2020
Received in revised form
23 May 2020
Accepted 26 May 2020
Available online 30 May 2020

Keywords:
Alzheimer's disease
Transcranial magnetic stimulation
Mild cognitive impairment
Memory
Long term potentiation
Biomarker
TMS

ABSTRACT

Background: New diagnostic criteria consider Alzheimer's disease (AD) as a clinico-biological entity identifiable in vivo on the presence of specific patterns of CSF biomarkers.
Objective: Here we used transcranial magnetic stimulation to investigate the mechanisms of cortical plasticity and sensory-motor integration in patients with hippocampal-type memory impairment admitted for the first time in the memory clinic stratified according to CSF biomarkers profile.
Methods: Seventy-three patients were recruited and divided in three groups according to the new diagnostic criteria: 1) Mild Cognitive Impaired (MCI) patients (n = 21); Prodromal AD (PROAD) patients (n = 24); AD with manifest dementia (ADD) patients (n = 28). At time of recruitment all patients underwent CSF sampling for diagnostic purposes. Repetitive and paired-pulse transcranial magnetic stimulation protocols were performed to investigate LTP-like and LTD-like cortical plasticity, short intracortical inhibition (SICI) and short afferent inhibition (SAI). Patients were the followed up during three years to monitor the clinical progression or the conversion to dementia.
Results: MCI patients showed a moderate but significant impairment of LTP-like cortical plasticity, while ADD and PROAD groups showed a more severe loss of LTP-like cortical plasticity. No differences were observed for LTD-like cortical plasticity, SICI and SAI protocols. Kaplan-Meyer analyses showed that PROAD and MCI patients converting to dementia had weaker LTP-like plasticity at time of first evaluation.
Conclusion: LTP-like cortical plasticity could be a novel biomarker to predict the clinical progression to dementia in patients with memory impairment at prodromal stages of AD identifiable with the new diagnostic criteria based on CSF biomarkers.

Introduction

Alzheimer's disease (AD) represents a complex pathophysiological process. It has been calculated that incidence and prevalence of AD will persistently increase worldwide [1]. Given this dramatic scenario, there have been efforts for a cultural shift in diagnosing AD at earlier stages, before patients crossed the dementia threshold.

Recently, with the introduction of biomarkers able to reflect in vivo the neuropathologic alterations occurring in the disease, substanstial modifications have been posed to AD definition giving birth to two sets of diagnostic criteria, those published by an International Working Group (IWG) in 2007 [2], and those by the National Institute on Aging and Alzheimer's Association (NIA-AA) [3]. Crucially, IWG and NIA-AA classification introduced the concept of prodromal AD (or Mild Cognitive Impairmetn [MCI] due to AD) stage allowing the identification in vivo of an underlying abnormal pathology in patients with an hippocampal-type memory impairment, even in absence of a global cognitive compromission; thus,

Abbreviations: AD, Alzheimer's Disease; ADD, Alzheimer's Disease Dementia; cTBS, continuous Theta-Burst Stimulation; iTBS, intermittent Theta-Burst Stimulation; IWG, International Working Group; LTD, long-term depression; LTP, long-term potentiation; MCI, Mild Cogntive Impairment; NIA-AA, National Institute on Aging and Alzheimer's Association; PROAD, Prodromal Alzheimer's Disease; rTMS, repetitive Transcranial Magnetic Stimulation; CSF, cerebrospinal fluid; RAVLT, Rey Auditory Verbal Learning Test.

* Corresponding author. Non Invasive Brain Stimulation Unit, Laboratorio di Neurologia Clinica e Comportamentale, IRCCS Fondazione S. Lucia, Via Ardeatina, 306, 00179, Rome, Italy.
E-mail address: g.koch@hsantalucia.it (G. Koch).

https://doi.org/10.1016/j.brs.2020.05.013

no more reference to the dementia threshold is needed for AD diagnosis [4]. Notwithstanding this diagnostic development, AD clinical course remains heterogeneous and unpredictable, mainly due to a scarce comprehension of the underlying pathophysiological mechanisms determining the severity of disease progression. Loss of synaptic density is an early event preceding neuronal degeneration, suggesting that synaptic plasticity mechanisms' impairment plays a key role in AD pathogenesis [5–7]. Notably, the strongest statistical correlation has been found between the loss of synaptic density and the degree of cognitive impairment in AD [8]. Thus, synaptic transmission impairment due to toxic oligomeric species [9] can predict disease severity more precisely than gross neuronal loss - a more tardive event - therefore placing synaptic dysfunction process as a key driver of AD-related cognitive decline rather than a mere byproduct.

In humans, neurophysiological techniques such as transcranial magetic stimulation (TMS) can be useful to predict AD disease progression by providing an estimate of cortical functioning at a certain time [10]. Particularly, cortical plasticity mechanisms such as long-term potentiation (LTP), the main neurophysiological correlates for learning and memory [11], can be assessed reliably and safely by means of non-invasive repetitive TMS (rTMS) [12]. In a series of previous works, we showed that AD patients have a consistent impairment of LTP-like cortical plasticity evaluated with intermittent theta burst stimulation (iTBS), a brief high-frequency rTMS protocol, applied over the primary motor cortex [13]. Oppositely, AD patients show spared long-term depression (LTD)-like cortical plasticity assessed with the continous TBS (cTBS) protocol. Moreover, we observed that such remarkable impairment of LTP-like cortical plasticity is independent from age of disease onset [14] and it is associated to a more aggressive clinical course [15]. These results are consistent with experimental studies showing that Aβ peptides and tau proteins interfere with physiological mechanisms of neuronal synaptic plasticity in AD animal models [16–20].

In the current work, we aimed at investigating the neurophysiological fetaures of LTP and LTD-like cortical plasticity in patients with memory decline at early stages of cognitive impairment, including MCI, prodromal AD (PROAD) and moderate AD dementia (ADD) patients, diagnosed according to the new criteria proposed for AD [2,3]. Patients were followed up to three years to explore how baseline neurophysiological characteristics may predict clinical progression.

Methods

Study participants

Participants to this study were recruited at the memory clinic of the University Hospital Tor Vergata (Rome, Italy), admitted for complaining memory symptoms between April 2010 and February 2014. Seventy-three consecutive patients (range, 55–80 years; median, 69) were recruited. Patients were divided in three groups according to the new diagnostic criteria IWG [2,3]: Mild Cognitive Impairment (MCI) patients (negative CSF biomarker and absence of dementia) (n = 21); Prodromal AD (PROAD) patients (positive CSF biomarkers and absence of dementia) (n = 24) and AD Dementia (ADD) patients (positive CSF biomarkers and presence of dementia) (n = 28). The three groups did not differ in gender, education, age at disease onset, disease duration, and ApoE genotype as shown in Table 1.

After the first visit, all subjects underwent for diagnostic purposes a clinical investigation including CT/MRI, and a complete neuropsychological assessment [21]. Once preliminary investigations were completed and treatable/non neurodegenerative causes of amnestic symptoms excluded, patients underwent a lumbar puncture for CSF for diagnostic purposes [22]. Thus, just after performing CSF sampling, subjects who showed an hippocampal-type memory impairment as assessed by RAVLT [23], were asked to participate to this study. Patients had not been treated six months before enrollment with antipsychotics, antiparkinsonian, anticholinergics and antiepileptic drugs. ADD patients were under treatment with acetylcholinesterase inhibitors. Neurophysiological examinations were performed at the Santa Lucia Foundation within 60 days from CSF sampling. Patients and examiners were blinded to the CSF profile. Thirty-three age-, sex-, and education-matched healthy subjects (HS) (range, 59–75 years; median, 67) were recruited as a control group for TMS evaluation. RAVLT scale was performed in HS to exclude subclinical impairment in hippocampal-type memory impairment. 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.

Biomarkers collection and genotype analysis

The first 12 mL of CSF were collected in a polypropylene tube and directly transported to the local laboratory for centrifugation at 2000g at +4 °C for 10 min [22]. 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 β-amyloid; Innogenetics), specifically constructed to measure Aβ-amyloid containing both the first and 42nd amino acid. Genotyping for APOE (locus 19q13.2) were performed by allelic discrimination technology (TaqMan; Applied Biosystems)^2 [24]. CSF biomarker positivity was calculated by using T-tau/Aβ1–42 ratio [25] and P-tau181/Aβ1–42 ratio [26] that are CSF predictors of conversion to dementia. Subjects who had a CSF T-tau/Aβ1–42 ratio >1.15 and P-tau181/Aβ1–42 ratio ≥0.214 were considered having evidence of AD pathology in vivo.

TMS

TMS recordings were performed at the Santa Lucia Foundation IRCCS (Rome, Italy) within three months from CSF examination. All patients and HS underwent cTBS, iTBS, short intracortical inhibition (SICI) and short-latency afferent inhibition (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 HS. All subjects were tested at the same time of day. Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle using Ag/AgCl surface cup electrodes. A monophasic Magstim 200 device 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 TMS pulse produced the largest MEP size at 120% of resting motor threshold in the target muscle [27]. A second coil was connected to a biphasic Super Rapid Magstim stimulator to deliver TBS [12]. TMS intensity was set at the 120% of the RMT at baseline and post-measures. Twenty MEPs were recorded for each time bin (1, 10, 20 min after TBS). The after effects of TBS were calculated on MEP peak-to-peak amplitude expressed as percentage of change in comparison to baseline for each TBS protocol.

SICI and ICF were studied at rest via a paired-pulse paradigm, delivered in a conditioning test design with the conditioning stimulus (CS) set at an intensity of 80% of the AMT, whereas the test stimulus (TS) was adjusted to evoke an MEP of approximately 1 mV peak to peak in the relaxed FDI. Different interstimulus intervals (ISIs) between the CS and TS were employed to investigate

Table 1
Demographic and clinical characteristic of subjects enrolled in the study.

	AD (n = 28)	PROAD (n = 24)	MCI (n = 21)	HS (n = 32)	p value
Age at baseline, y (mean ± SD)[a]	36.8 ± 5.7	70.2 ± 6.2	66 ± 6.4	66.2 ± 5.1	n.s.
Female (%)[b]	50%	54%	59%	44%	n.s.
Disease duration, m (mean ± SD)[a]	13.1 ± 3.5	13.2 ± 2.9	15.1 ± 4.1	–	n.s
Education, y (mean ± SD)[a]	8.3 ± 3.7	8.3 ± 3.1	9.4 ± 4.6	8.2 ± 3.4	n.s
CSF total-tau pg/ml (mean ± SD)[a]	830.1 ± 372	794.1 ± 245	296.5 ± 118	–	<0.001*
CSF p-tau pg/ml (mean ± SD)[a]	98.3 ± 52	84.2 ± 36	49.1 ± 21	–	<0.001*
CSF beta 1–42 pg/ml (mean ± SD)[a]	315.6 ± 114	276.7 ± 136.1	605.2 ± 272	–	<0.001*
APOE4 (E3/E4 + E4/E4) (%)[b]	35%	30%	30%	–	n.s
MMSE baseline (mean ± SD)[a]	18.9 ± 2.5	25.4 ± 1.2	25.8 ± 2.09	29.5 ± 0.5	<0.001^

* = significant difference between MCI group and both PROAD and ADD groups. ^ = significant difference betwenn ADD group and PROAD and MCI groups. Kruskal-Wallis analysis of variance was performed for analyzing the difference among the 3 independent groups, followed by Mann-Whitney *U* test with Bonferroni correction when significant. Chi-2 Test was used for categorical variables. Pairwise comparisons of 2 related samples were made by the Wilcoxon matched-pairs signed ranks test. CSF = cerebrospinal fluid; MMSE = Mini–Mental State Examination; ADD = Alzheimer disease dementia; HS = healthy subjects; MCI = Mild Cognitive Impairment; PROAD=Prodromal Alzheimer.

[a] Kruskal-Wallis test.

[b] Chi-2 test.

preferentially both SICI (1, 2, 3, and 5 ms) and ICF (7, 10, and 15 ms). The amplitude of the conditioning MEPs was expressed as a ratio of the mean unconditioned response [28]. SAI, which primarily reflects cholinergic transmission, was studied using a previously described technique [29]. CSs were single pulses (200 μs) of electrical stimulation applied through bipolar electrodes to the right median nerve at the wrist (cathode proximal). The intensity of the CS was set at just over motor threshold for evoking a visible twitch of the thenar muscles, whereas the TS was adjusted to evoke an MEP of approximately 1 mV peak to peak. The CS to the peripheral nerve preceded the TS by different ISIs (−4, 0, +4, +8 ms, determined relative to the latency of the N20 component of the somatosensory evoked potential). Ten stimuli were delivered for each ISI for all stimulation paradigms, and fourteen control MEPs in response to the TS alone, were recorded for each paradigm in all participants in a pseudorandomized sequence. The amplitude of the conditioning MEPs was expressed as a ratio of the mean unconditioned response. The intertrial interval was set at 5 s (±10%).

Statistical analysis

Data were analysed using SPSS for Windows (version 11.0; SPSS, Inc., Chicago, IL). Data were first tested for normality using Kolmogorov-Smirnov (K-S) test. For each ANOVA, Mauchley's test was used to test sphericity data; for non spherical data we used the Greenhouse-Geisser correction. For TMS measures, two-way repeated-measures ANOVAs were performed on MEP peak-to-peak amplitude expressed as percentage of change in comparison to baseline for each TBS protocol (cTBS and iTBS) with time (1, 10, and 20 min after TBS) as within-subject factors and group (ADD, PROAD, MCI and HS) as between-subjects factor. For SICI, the electrophysiological parameters were compared by means of repeated-measures ANOVA with ISI (1,2,3,5,7,10,15 ms) as within-subject factor and group. For SAI, the electrophysiological parameters were compared by means of repeated-measures ANOVA with ISI (−4, 0, +4, +8 ms plus the latency of the N20) and group (ADD, PROAD, MCI and HS). When a significant main effect was reached, paired or unpaired *t*-test with Bonferroni's correction were used for post-hoc comparisons to characterize the different effects of the specific time-points or ISIs. Kaplan-Meier survival analysis was used to examine the distribution of cognitive decline from baseline until a defined endpoint (36 months) in the MCI and PROAD group. Breslow test was performed to analyze the equality of the survival distributions for the different groups. Multivariate Cox proportional hazards regression models were used to estimate the effects of LTP-like cortical plasticity on the relative hazard of cognitive decline within 36 months.

Responders and non responders to iTBS were classified as having a mean change of MEP amplitude (calculated as the percentage of change of all MEP amplitudes recorded after TBS respect to baseline) > 100% or <100% respectively [13,15]. A p value of <0.05 was considered statistically significant.

Data availability

The data used in this manuscript along with related documents such as study protocol, and statistical analysis will be shared on request from any qualified investigator for 3 years after the date of publication.

Results

TMS

The procedure was well tolerated by all subjects. RMT (mean ± standard deviation [SD]) was lower in ADD and PROAD patients, but not MCI, in comparison to HS (respectively $p = 0.009$ and $p = 0.01$) (ADD: 36.1 ± 1.14%; PROAD: 37.4 ± 1.89; MCI: 40.8 ± 2.74; HS: 44.5 ± 3.49%; $p = 0.003$). ANOVA performed on baseline mean MEP amplitude did not show any difference between the four groups across all protocols (AD: 1.14 ± 0.38 mV; PROAD: 1.13 ± 0.41; MCI: 1.15 ± 0.52; HS: 1.13 ± 0.42 mV).

Fig. 1 shows the distribution of changes in plasticity after iTBS and cTBS protocol in the for MCI, PROAD, ADD and HS. Analysis of iTBS showed a main effect for group ($F(3,90) = 15.69$; $p < 0.0001$) but not for time ($F(2,180) = 0.39$; $p = 0.67$); the group × time interaction was significant ($F(6,180) = 4.43$; $p < 0.0001$). Post-hoc analysis showed that AD and PROAD patients differed from HS at T10, and T20 (all $p < 0.001$; Fig. 2A); post-hoc analysis revealed also a difference between MCI and HS at T10 ($p = 0.01$) and a difference between MCI and both ADD and PROAD at T20 (respectively $p = 0.03$ and $p = 0.04$). In ADD and PROAD groups the mean response to iTBS turned from LTP- to LTD-like plasticity, according to previous findings showing a similar reversal of LTP in AD [15].

Analysis of cTBS ANOVA revealed no main effect of group($F(3,90) = 1.30$; $p = 0.28$) or time ($F(2,180) = 1.61$; $p = 0.20$), and no significant group × time interaction ($F(6,180) = 0.85$; $p = 0.52$) (Fig. 2B).

Analysis of SICI showed a main effect of ISI ($F(6,366) = 27.41$; $p=<0.0001$), but not of group ($F(3,61) = 0.65$; $p = 0.58$), nor for group × ISI interaction ($F(18,366) = 1.07$; $p = 0.37$) (Fig. 2C).

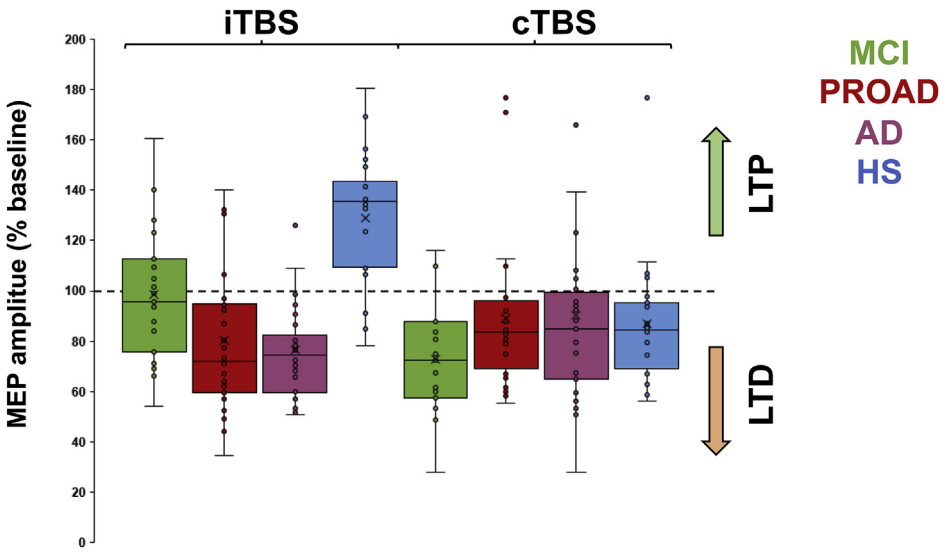

Fig. 1. Distribution of changes in MEP amplitude after iTBS and cTBS protocols in MCI, PROAD, AD and HS.

Analysis of SAI showed a main effect of ISI ($F(3,270) = 48.57$; $p < 0.0001$) but not of group ($F(3,90) = 1–39$; $p = 0.25$), nor for group × ISI interaction ($F(9,270) = 0.67$; $p = 0.73$). (Fig. 2D).

Clinical follow-up

After the neurophysiological assessment, PROAD and ADD were all treated with standard acetylcholinesterase inhibitor (AchEI) therapy (rivastigmine/donepezil). The two groups did not differ in the number of patients undergoing diferent AchEI treatment (PROAD: 12 patients under rivastigmine and 12 patients under donepezil treatment; ADD: 15 patients under rivastigmine and 13 patients under donepezil treatment; $p = 0.81$). We followed patients up to a period of 36 months with clinical testing every 6 months.

Follow-up evaluation, with MMSE scores [30] performed at 6, 12, 18, 24, 30 and 36 months, revealed that ADD and PROAD patients progressed faster than MCI patients as shown by ANOVA (significant group × time interaction ($F(10,26) = 7.64$, $p < 0.0001$)). Indeed, post-hoc analyses revealed that MMSE scores of ADD and PROAD patients, were lower than baseline evaluation as early as at 12 months ($p = 0.01$ and $p = 0.05$ respectively), while MCI patients'

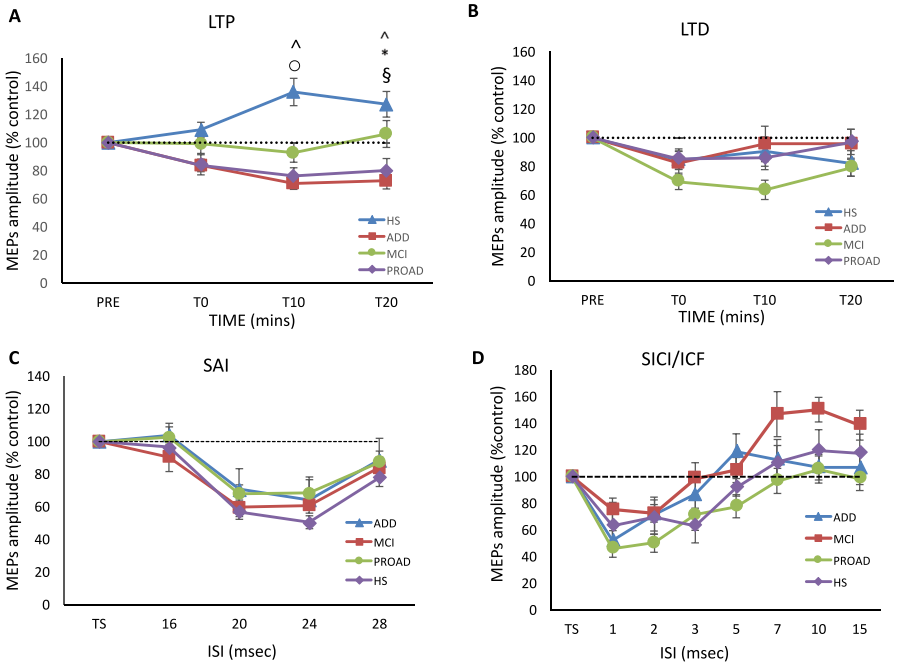

Fig. 2. A) After effects of iTBS protocol on MEP amplitude in ADD, PROAD, MCI and HS. B) After effects of cTBS protocol on MEP amplitude in ADD, PROAD, MCI and HS. Response to iTBS turned from LTP- to LTD-like plasticity in ADD and PROAD groups. C) Changes in MEP amplitude for the SAI protocol in ADD, PROAD, MCI and HS. D) Changes in MEP amplitude for the SICI/ICF protocol in ADD, PROAD, MCI and HS Error bars indicate standard error of the mean. * = $p < 0.05$ between HS and PROAD; ^ = $p < 0.05$ betwenn HS and ADD; § = $p < 0.05$ betwenn MCI and both PROAD and ADD; ○ = $p < 0.05$ betwenn MCI and HS. ADD = Alzheimer's disease dementia; cTBS = continuous theta burst stimulation; HS = healthy subjects; MCI = Mild Cognitive Impairment; MEP = motor evoked potential; PROAD=Prodromal Alzheimer's disease dementia.

MMSE scores became significantly lower respect to baseline only after 36 months ($p = 0.03$) (Table 2).

Subsequently, we looked how many patients from the PROAD and MCI group evolved to a stage of dementia at 36 months follow-up. 79.1% (19 patients on 24) of PROAD patients and 57,1% (11 patients on 21) of MCI patients converted to a state of dementia. Then, we wanted to investigate which were the differences between the converters and the non-converters for each group (PROAD and MCI). As shown in Table 3 the only significant differences among converters and non-converters for both groups were observed in terms of LTP-like plasticity (and, as expected, in terms of clinical progression [31,32]).

The Breslow analysis of the distribution of overall cognitive decline from baseline to 36 months is depicted in Fig. 3. This analysis showed a higher percentual of dementia conversion in PROAD group compared to MCI [$\chi^2(1) = 4.14$; $p = 0.042$] (Fig. 3). Then, since the conversion to a dementia state in both group of patients seems associated to the impairment to LTP-like cortical plasticity, we conducted a specific Kaplan-Meyer survival analysis for PROAD and MCI groups dividing patients according to their iTBS after-effects in those who were showing an impaired LTP-like plasticity (<100% intended as percentage of change of MEPs amplitude after iTBS in comparison to baseline MEPs) and those with normal LTP-like plasticity (>100% of mean MEP amplitude change following iTBS). The threshold for “responders" and "non-responders” was set on the basis of previous literature [33,34]. Kaplan-Meier analysis revealed that both MCI [$\chi^2(1) = 4.404$; $p = 0.036$] and ADD [$\chi^2(1) = 8.904$; $p = 0.003$] patients with impaired LTP-like plasticity had a more aggressive clinical course (Fig. 4). Multivariate Cox proportional hazards models revealed that for both groups patients who had an impaired LTP-like had a significant higher risk of cognitive decline than patients with an unimpaired LTP-like (for PROAD patients: hazard ratio 3.7, 95%, confidence interval [CI] 0.6–1.8; for MCI patients hazard ratio 4.8, 95% CI 0.9–2.1), even after adjustment for age and gender, baseline MMSE score, and APOE genotype.

Discussion

By using new diagnostic criteria taking in account for the in vivo evidence of AD pathology biomarkers, neurophysiological evaluation allowed us to shed light on physiopathological mechanisms of clinical progression in patients with cognitive impairment at different stages of disease. Our findings show that i) MCI patients fail to have the physiological LTP-like after-effects in comparison to HS; ii) PROAD patients show a more robust impairment of LTP-like cortical plasticity similarly to ADD patients; iii) approxymately 80% of PROAD patients and half of MCI patients converted after 36 months to a a frank state of dementia; iv) PROAD and MCI patients converting to dementia have weaker LTP-like plasticity at time of first evaluation.

In this study we extend previous knowledge showing that LTP-like cortical plasticity represents a key neurophysiological marker of cortical dysfunction in AD patients, even when evaluated at a pre-dementia state. According to previous work in animal models, the onset of memory deficits in AD is not due to neuronal loss [35,36] but is more closely related to the loss of functional synapses [9] expecially in the hippocampus, due to a relevant vulnerability of the glutamatergic synapses [37–40]. Indeed, synaptic loss is the best morphological correlate of cognitive impairment in early AD, rather than amyloid-beta plaques, tangle formation or neuronal loss [9]. Consistently, loss of episodic hippocampal-dependent memory is the earliest clinical sign of AD [1]. In this context, neuropsyhological tests investigating hippocampal function, such as RAVLT [21,41], have shown a tight association of hippocampal efficiency with brain atrophy [42]. Moreover neurophysiological recordings showed that deposition of Aβ peptides [17] or tau proteins [15,16,43] are able to impair mechanisms of hippocampal LTP. Therefore, it is widely recognised that impairment of hippocampal synaptic plasticity is the main cause of memory loss in the early stage of AD [44], mainly due to the disruption of synaptic plasticity mechanisms. In humans, similar mechanisms of sinaptic plasticity can be investigated non-invasively by applying rTMS over the motor cortex [12] paralleling the neurophysiological findings assessed experimentally on hippocampal slices, both in healthy subjects [12] and in AD patients [13]. Compromission of mechanisms of LTP-like cortical plasticity assessed with iTBS on motor cortex resembles the impairment of hippocampal plasticity assessed in AD animal models [14]. In healthy subjects, the evaluation of motor cortex plasticity shows a large interindividual variability [45]; on the other hand iTBS after-effects are more reproducible in AD patients than age-matched healthy controls, reflecting a pathological rigidity of the impaired neurophysiological system [46].

Our groups of patients were completely undistinguishable for demographic variables, genetic risk (apoe4 distribution across groups), vascular risk factors (diabetes, hypertension, hypercholesterol), clinical onset (all amnestic forms), and disease duration. This means that the differences in cognitive performances observed at baseline cannot be regarded as expression of different disease stages, but rather reflect a different underlying pathology. This is in line with previous studies showing that AD patients with relatively high levels of t-tau and p-tau and low levels of A-beta 1–42 have a faster clinical progression [31].

Moreover, we did not find any clear-cut differencies in response to SAI protocol. The impairment of cholinergic transmission evaluated by SAI has been one of the first consistent TMS findings in patients with AD and it is clinically useful to discriminate between different forms of dementia [47]. In the current study, the absence of differences among the different groups in terms of sensory-motor integration investigated with SAI protocol is likely explained by our previuos observation showing in a large population of AD patients and age matched controls that cholinergic impairment is strongly influenced by a general mechanism of ageing, not entirely depending on the underlying AD pathology, while LTP-like cortical plasticity impairment is associated with disease progression [14].

Our findings are in line with this conceptual framework, showing that both ADD (patients with memory and functional demise) and PROAD (patients complaining only memory deficit

Table 2
Clinical Progression in the different groups of patients.

	Baseline	6 months	12 months	18 months	24 months	30 months	36 months
MCI	26.1 ± 1.0	25.7 ± 0.4	25.2 ± 0.6	24.8 ± 0.6	24.5 ± 0.6	24 ± 0.7	23.3 ± 0.8*
PROAD	25.4 ± 1.2	24.5 ± 0.5	23 ± 0.8 *	22.5 ± 0.9*	21.5 ± 0.9*	20.5 ± 0.9*	19.5 ± 0.9*
ADD	18.9 ± 2.5	18.0 ± 0.7	16.4 ± 0.9*	15 ± 1.0*	14 ± 1.0*	12 ± 1.0*	11 ± 0.9*

Clinical progression calculated with MMSE in ADD, MCI and PROAD patients. ADD = Alzheimer's disease dementia; MCI = Mild Cognitive Impairment; PROAD= Prodromal Alzheimer. * = $p < 0.05$ compared to baseline MMSE.

Table 3
Demografic, clinical and neurophisological differences between MCI converters and MCI non-converters and between PROAD converters and non-converters.

	MCI converter (n = 11)	MCI non converter (n = 10)	*p* value	PROAD converter (n = 19)	PROAD non converter (n = 5)	*p* value
Age at baseline, y (mean ± SD)[a]	65.3 ± 6.8	66.8 ± 5.9	0.59	70.23 ± 6.4	70.2 ± 6.1	0.98
Female (%)[b]	54%	60%	0.67	58%	40%	0.97
Disease duration, m (mean ± SD)[a]	14.7 ± 4.4	15.9 ± 6.6	0.24	13.14 ± 4.2	13.66 ± 6.2	0.78
Education, y (mean SD)[a]	9.3 ± 4.1	8.6 ± 4.6	0.49	8.8 ± 4.2	9.2 ± 4.7	0.49
CSF total-tau pg/ml (mean ± SD)	308.6 ± 116.4	283.2 ± 125.1	0.63	821.9 ± 284.6	688.6 ± 312.3	0.28
CSF p-tau pg/ml (mean ± SD)	65.8 ± 28.5	55.8 ± 23.6	0.29	81.42 ± 39.3	94.8 ± 28.7	0.48
CSF beta 1–42 pg/ml (mean ± SD)	628.3 ± 305.1	579.8 ± 245.5	0.69	276.0 ± 146.7	279.4 ± 102.6	0.96
APOE4 (E3/E4 + E4/E4) (%)[b]	27%	30%	0.57	45%	40%	0.93
MMSE	25.96 ± 1.1	26.5 ± 1.0	0.26	25.8 ± 0.9	25.4 ± 0.8	0.55
MMSE 36 months	19.36 ± 0.61	25.26 ± 0.75	<0.001*	19.30 ± 1.13	24.9 ± 0.23	0.009*
LTP plasticity (% mean ± SD) [c]	83.9 ± 5.1	114.8 ± 9.2	0.007*	69.2 ± 6.81	121.4 ± 14.78	<0.001*
LTD plasticity (% mean ± SD) [c]	75.8 ± 6.14	81.6 ± 10.38	0.59	98.6 ± 8.34	83.7 ± 9.64	0.49
SAI + 4 ms (% mean ± SD)[c]	57.2 ± 29.43	61.3 ± 21.76	0.43	66.4 ± 33.67	70.4 ± 28.45	0.60
SICI 3 ms (% mean ± SD) [c]	100.0 ± 18.3	97.2 ± 21.4	0.92	68.7 ± 22.45	71.4 ± 26.49	0.69
ICF 15 ms (% mean ± SD) [c]	156.4 ± 30.3	143.2 ± 27.4	0.79	99.2 ± 31.24	96.7 ± 28.91	0.91
RMT (% mean ± SD)[d]	42.4 ± 5.7	38.5 ± 4.6	0.27	37.1 ± 4.1	39.6 ± 5.8	0.68

[a] Student *t*-test.
[b] Fisher's exact test.
[c] Related to the control MEP amplitude.
[d] Related to the maximal stimulator output. For SICI, ICF, SAI, LTP and LTD values, '%' are related to the control MEP amplitude. * = p < 0.05. CSF = cerebrospinal fluid; MMSE = Mini–Mental State Examination; MCI = Mild Cognitive Impairment; ICF = intracortical facilitation; LTP = long-term potentiation; MEP = motor evoked potential; RMT = resting motor treshold; SAI, short-latency afferent inhibition; SICI = short intracortical inhibition.

with still spared everyday abilities) groups show the same impairment of LTP-like cortical plasticity mechanisms and the same rate of cognitive decline, independently from the age of onset [14].

These findings are important due to the increasing need that treatment options should be evaluated at earlier disease stages before the full picture of dementia is reached [46,47]. The follow up analysis in PROAD and MCI groups showed that patients who progressed to a dementia state in a 3-year time window had more impaired LTP-like cortical plasticity related to the patients who did not, while no difference was observed for SAI and SICI. Moreover, these data highlight the prognostic role of LTP-like cortical plasticity evaluation [10,13–15] in patients with memory impairment even in those where the evidence of AD biomarker is absent or uncertain. Nonetheless, we have to consider that the sensitivity and specificity of the sole clinical criteria in making a diagnosis of AD are widely recognised to be imperfect [3,4,46–48]. Similarly, the application of new criteria by using imaging and CSF biomarkers pointed out different combinations of biomarkers profiles in the ageing brain with and without cognitive impairment [3]. The absence of primary amiloyd pathology however does not exclude a possible neurodegeneration, going also beyond the classical amyloid cascade hypothesis. For instance, current definition of neurodegeneration entails different pathological situations, endowed under the Suspected Non Alzheimer Pathology (SNAP) definition [49]. SNAP condition has been related to hippocampal sclerosis, primary age-related tauopathy or strategic microvascular lesions in memory networks [50]. The use of a single biomarker could be misleading in distinguishing AD and non-AD pathology. Multiple biomarkers investigating different pathophysiological mechanisms might help in identifying the underlying pathology and, more importantly, help to predict clinical progression. In this view, the introduction of a new tool able to predict clinical progression on the basis of the evaluation of a reliable neurophysiological parameter such as LTP-like cortical plasticity in patients with memory impairment could be helpful to estimate possible conversion to dementia in these uncertain conditions.

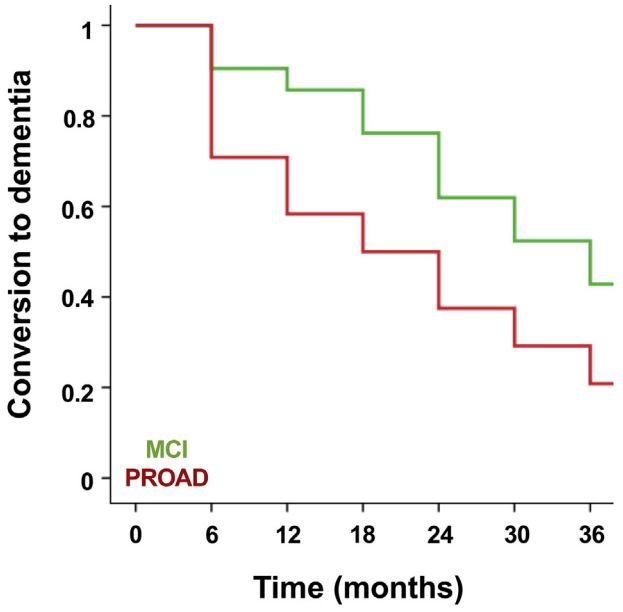

Fig. 3. Kaplan-Meier survival analysis of overall cognitive decline in MCI and PROAD patients. Green line depicts the progression of MCI patients, red line depicts the progression of PROAD patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

A limitation of the current study is that we did not detect AD biomarkers with specific beta-amyloid or tau PET tracers but only with CSF analysis using the t-tau/Aβ1–42 ratio [28] and p-tau181/Aβ1–42 ratio [29]; thus we were not able to recognize pathological accumulation of other misfolding proteins. Moreover, when performing the Kaplan-Meyer analysis based on the reponse to iTBS protocol, we allocated AD patients into "responders" and non-responders" groups. However, we chose an arbitrary cut-off of >100% of mean MEP amplitude change following iTBS for "responders" and of <100% of mean MEP amplitude change following iTBS for "non-responders" that needs to be validated in larger populations of AD patients. Finally, our study was performed in a limited sample of subjects recruited in a single center and thus might be replicated in the context of a larger multicenter study [48].

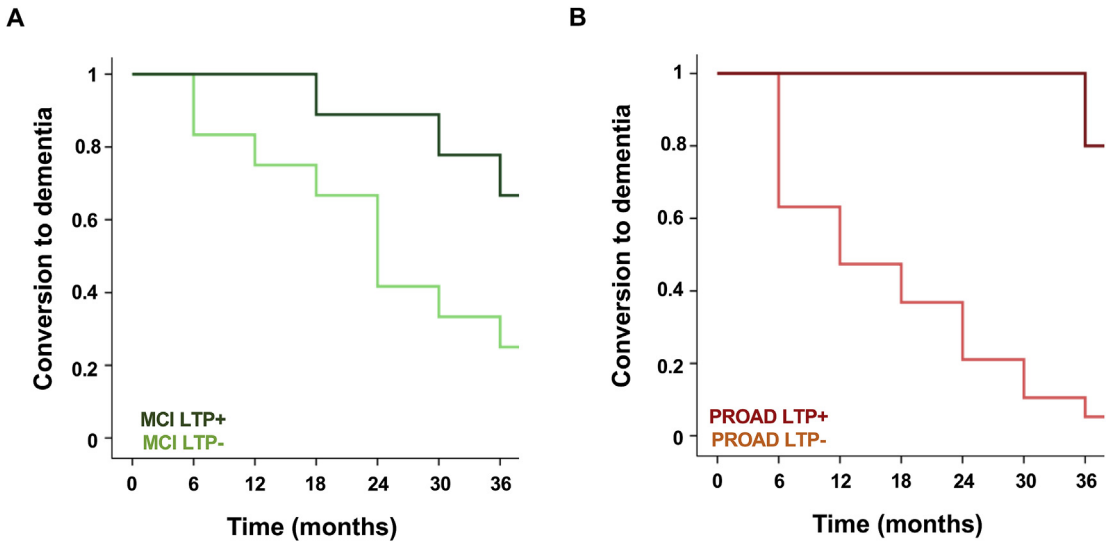

Fig. 4. Kaplan-Meier survival analysis in MCI (panel A) and PROAD patients (panel B) dividing patients from each group according to their iTBS after effects in who was showing an impaired LTP plasticity ("non-responders to iTBS" less than 100% intended as percentage of change of MEPs amplitude after iTBS in comparison to baseline MEPs, respectively MCI- and PROAD-) and who was showing an unimpaired LTP plasticity ("responders to iTBS" more than 100%, respectively MCI+ and PROAD+).

Conclusions

In conclusion, our findings suggest that neurophysiological investigations based on TMS methods, such as iTBS-induced LTP-like cortical plasticity, can provide in a non-invasive manner important information on possible conversion to dementia in patients with early memory impairment. Indeed, ouw work suggest that a reduced level of LTP-like cortical plasticity may represent a new biomarker for risk of developing AD, an hypothesis that is worth to assess in future studies in large populations of aged normal subjects. Our data reinforce the notion that TMS could be used as a biomarker to characterize brain circuits early involved in dementia and to identify changes predictive of progression and/or response to treatments [10]. Being able to select patients with an expected slower or faster cognitive decline is of major interest for assessing the efficacy of new AD therapies and for stratifying clinical trial cohorts [51]. Moreover, our results may pave the way for the identification of new therapeutic targets. Large clinical trials testing drug reducing protein toxicity in AD are not giving the expected positive results. On the other hand, synaptic modulators, such as those acting on the dopaminergic and serotoninergic system, are considered promising targets to alleviate cognitive dysfunction in early AD patients [52]. In this perspective, our data support the hypothesis that preventing synapse loss and improving synaptic function may be considered a promising therapeutic approach to counteract the cognitive impairment in AD pathology.

Author contributions

1) FDL, CC, AM, and GK contributed to the design of the study, 2) FDL, CM, EPC, MA and SB acquired and analysed of data or 3) FDL, EPC and GK drafted the manuscript and figures.

Funding

This work was partially supported by grants of the Alzheimer's Drug Discovery Foundation (ADDF) (n°20150502), the Brighfocus Foundation (A2019523S) to GK, and the Italian Ministry of Health (GR-2016-02364718) to SB.

Declaration of competing interest

All the authors report no conflicts of interest relevant to the manuscript.

The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

CRediT authorship contribution statement

Francesco Di Lorenzo: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Caterina Motta:** Data curation, Formal analysis, Methodology. **Elias Paolo Casula:** Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Sonia Bonnì:** Data curation, Formal analysis, Methodology. **Martina Assogna:** Data curation, Formal analysis, Methodology. **Carlo Caltagirone:** Conceptualization. **Alessandro Martorana:** Conceptualization. **Giacomo Koch:** Conceptualization, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.05.013.

References

[1] Cummings JL. Alzheimer's disease. N Engl J Med 2004;351:56–67.
[2] Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS–ADRDA criteria. Lancet Neurol 2007;6:734–46.
[3] Jack Jr CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14:535–62.
[4] Dubois B, Feldman HH, Jacova C, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol 2010 Nov;9(11):1118–27.
[5] Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. Neurobiol Aging 2003 Dec;24(8):1029–46.
[6] Walsh DM, selkoe DJ. Deciphering the molecular basis of memory failure in alzheimer's disease. Neuron 2004;44:181–93.

[7] Ingelsson M, Fukumoto H, Newell KL, et al. Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology 2004;62:925–31.
[8] Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30:572–80.
[9] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science 2002;298:789–91.
[10] Motta C, Di Lorenzo F, Ponzo V, et al. Transcranial magnetic stimulation predicts cognitive decline in patients with Alzheimer's disease. J Neurol Neurosurg Psych 2018;89(12):1237–42.
[11] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993:31–9.
[12] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–6.
[13] Koch G, Di Lorenzo F, Bonnì S, Ponzo V, Caltagirone C, Martorana A. Impaired LTP- but not LTD-like cortical plasticity in Alzheimer's disease patients. J Alzheimers Dis 2012;31:593–9.
[14] Di Lorenzo F, Ponzo V, Bonnì S, Motta C, Negrão Serra PC, Bozzali M, et al. Long-term potentiation-like cortical plasticity is disrupted in Alzheimer's disease patients independently from age of onset. Ann Neurol 2016;80:202–10.
[15] Koch G, Di Lorenzo F, Del Olmo MF, Bonní S, Ponzo V, Caltagirone C, et al. Reversal of LTP-like cortical plasticity in Alzheimer's disease patients with tau-related faster clinical progression. J Alzheimers Dis 2016;50:605–16.
[16] Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. Mol Neurodegener 2011 Jun 6;6:39.
[17] Selkoe DJ. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res 2008;192:106–13.
[18] Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci 2010;13:812–8.
[19] Klyubin I, Cullen WK, Hu N-W, Rowan MJ. Alzheimer's disease Aβ assemblies mediating rapid disruption of synaptic plasticity and memory. Mol Brain 2012;17:5–25.
[20] Llorens F, Schmitz M, Karch A, et al. Comparative analysis of cerebrospinal fluid biomarkers in the differential diagnosis of neurodegenerative dementia. Alzheimers Dement 2016 May;12(5):577–89.
[21] Di Lorenzo F, Motta C, Bonnì S, et al. LTP-like cortical plasticity is associated with verbal memory impairment in Alzheimer's disease patients. Brain Stimul 2019;12:148–51.
[22] Martorana A, Esposito Z, Di Lorenzo F, et al. Cerebrospinal fluid levels of Aβ42 relationship with cholinergic cortical activity in Alzheimer's disease patients. J Neural Transm (Vienna) 2012;119:771–8.
[23] Ricci M, Graef S, Blundo C, Miller LA. Using the Rey Auditory Verbal Learning Test (RAVLT) to differentiate Alzheimer's dementia and behavioural variant fronto-temporal dementia. Clin Neuropsychol 2012;26:926–41.
[24] Koch G, Di Lorenzo F, Loizzo S, et al. CSF tau is associated with impaired cortical plasticity, cognitive decline and astrocyte survival only in APOE4-positive Alzheimer's disease. Sci Rep 2017;7. 13728.
[25] Maddalena A, Papassotiropoulos A, Müller-Tillmanns B, et al. Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau protein to beta-amyloid peptide42. Arch Neurol 2003 Sep;60(9):1202–6.
[26] Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 2007 Mar;64(3):343–9.
[27] Rossini PM, Barker AT, Berardelli A, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 1994;91:79–92.
[28] Kujirai T, Caramia MD, Rothwell JC, et al. Corticocortical inhibition in human motor cortex. J Physiol 1993 Nov;471:501–19.
[29] Tokimura H, Di Lazzaro V, Tokimura Y, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000;523:503–13.
[30] Magni E, Binetti G, Padovani A, et al. The mini-mental state examination in alzheimers disease and multi-infarct dementia. Int Psychogeriatr 1999;8:127–34.
[31] Wallin AK, Blennow K, Zetterberg H, Londos E, Minthon L, Hansson O. CSF biomarkers predict a more malignant outcome in Alzheimer disease. Neurology 2010;74:1531–7.
[32] Koch G, Belli L, Lo Giudice T, et al. Frailty among Alzheimer's disease patients. CNS Neurol Disord - Drug Targets 2013;12:507–11.
[33] Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. Cerebr Cortex 2013;7:1593–605.
[34] Hinder MR, Goss EL, Fujiyama H, Canty AJ, Garry MI, Rodger J, et al. Inter- and Intra-individual variability following intermittent theta burst stimulation: implications for rehabilitation and recovery. Brain Stimul 2014;7:365–71.
[35] Citron M. Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov 2010;9:387–98.
[36] Heininger K. A unifying hypothesis of Alzheimer's disease. IV. Causation and sequence of events. Rev Neurosci 2000;11 Spec:213–328.
[37] Yang YJ, Zhao Y, Yu B, et al. GluN2B-containing NMDA receptors contribute to the beneficial effects of hydrogen sulfide on cognitive and synaptic plasticity deficits in APP/PS1 transgenic mice. Neuroscience 2016;335. 170-183.
[38] Viana da Silva S, Haberl MG. Zhang Pet al: early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A2A receptors. Nat Commun 2016;7:11915.
[39] Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 2006;27:1372–84.
[40] Canas PM, Simões AP, Rodrigues RJ, Cunha RA. Predominant loss of glutamatergic terminal markers in a β-amyloid peptide model of Alzheimer's disease. Neuropharmacology 2014;76:51–6.
[41] Landau SM, Harvey D, Madison CM, et al. Comparing predictors of conversion and decline in mild cognitive impairment. Neurology 2010;75:230–8.
[42] Moradi E, Hallikainen I, Hänninen T, Tohka J, Alzheimer's Disease Neuroimaging Initiative. Rey's Auditory Verbal Learning Test scores can be predicted from whole brain MRI in Alzheimer's disease. Neuroimage Clin 2016;13:415–27.
[43] Koch G, Esposito Z, Kusayanagi H, et al. CSF tau levels influence cortical plasticity in Alzheimer's disease patients. J Alzheimers Dis 2011;26:181–6.
[44] Morris GP, Clark IA, Vissel B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol Commun 2014;2:135.
[45] López-Alonso V, Cheeran B, Río-Rodríguez D, Fernández-Del-Olmo M. Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul 2014 May-Jun;7(3):372–80.
[46] Fried PJ, Schilberg L, Brem AK, et al. Humans with type-2 diabetes show abnormal long-term potentiation-like cortical plasticity associated with verbal learning deficits. J Alzheimers Dis 2017;55:89–100.
[47] Benussi A, Di Lorenzo F, Dell'era V, et al. Transcranial magnetic stimulation distinguishes alzheimer disease from frontotemporal dementia. Neurology 2017;89:665–72.
[48] Raskind MA, Peskind ER, Wessel T, Yuan W. Galantamine in AD: a 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group. Neurology 2000;54:2261–8.
[49] Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of alzheimer disease at National Institute on aging alzheimer disease centers, 2005-2010. J Neuropathol Exp Neurol 2012;71:266–73.
[50] Dani M, Brooks DJ, Edison P. Suspected non-Alzheimer's pathology - is it non-Alzheimer's or non-amyloid? Ageing Res Rev 2017;36:20–31.
[51] Benussi A, Grassi M, Palluzzi F, et al. Classification accuracy of transcranial magnetic stimulation for the diagnosis of neurodegenerative dementias. Ann Neurol 2020;87(3):394–404.
[52] Martorana A, Koch G. Is dopamine involved in Alzheimer's disease? Front Aging Neurosci 2014 Sep 25;6:252.