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Sensorimotor Cortex Excitability and Connectivity in Alzheimer's Disease: A TMS-EEG Co-Registration Study

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Abstract: Several studies have shown that, in spite of the fact that motor symptoms manifest late in the course of Alzheimer's disease (AD), neuropathological progression in the motor cortex parallels that in other brain areas generally considered more specific targets of the neurodegenerative process. It has been suggested that motor cortex excitability is enhanced in AD from the early stages, and that this is related to disease's severity and progression. To investigate the neurophysiological hallmarks of motor cortex functionality in early AD we combined transcranial magnetic stimulation (TMS) with electroencephalography (EEG). We demonstrated that in mild AD the sensorimotor system is hyperexcitable, despite the lack of clinically evident motor manifestations. This phenomenon causes a stronger response to stimulation in a specific time window, possibly due to locally acting reinforcing circuits, while network activity and connectivity is reduced. These changes could be inter-

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preted as a compensatory mechanism allowing for the preservation of sensorimotor programming and execution over a long period of time, regardless of the disease's progression. *Hum Brain Mapp 37:2083–2096, 2016*.

Key words: Alzheimer's disease; sensorimotor cortex excitability; sensorimotor cortex connectivity; EEG; navigated transcranial magnetic stimulation

INTRODUCTION

Alzheimer's disease (AD) is a progressive, disabling neurodegenerative disorder that is histopathologically defined by the presence of amyloid-β (Aβ) plaques and tau-related neurofibrillary tangles. These plaques and tangles have been associated with local synaptic disruption, leading not only to regional brain structural abnormalities but also to changes in neuronal functional connectivity between anatomically distinct brain regions, neuronal pools and circuitries [Arendt, 2009; Grutzendler et al., 2007]. This evidence complements the conception that AD is predominantly a dysconnection syndrome [Delbeuck et al., 2003] in which local alterations of complex networks can have widespread effects [He et al., 2009].

Motor symptoms are considered late events in the natural history of AD and their early occurrence makes the diagnosis less likely. The delayed involvement of the motor system has been variably explained. A smaller burden of neuropathological changes in the motor cortices than in other brain areas, a rich dendritic arborisation and a progressive neuronal reorganization compensatory for neural loss, have all been hypothesized [Suvà et al., 1999; for a review D'Amelio and Rossini, 2012]. Several neuropathological studies in animals and humans, though, have shown that the density of amyloid-β plaques and tau-related neurofibrillary tangles in the motor cortex (M1) is comparable to other cortices generally considered more specific 'early' targets for AD aggression, such as the enthorinal cortex, the hippocampus and the associative parietal and frontal areas [Golaz et al., 1992; Mastrangelo and Bowers, 2008; Suvà et al., 1999; Yuan et al., 2013]. Moreover, the motor cortex receives a major cholinergic input from the Nucleus Basalis of Meynert, one of the brain areas most affected by neuropathologic changes [Suvà et al., 1999]. The motor cortex is thus involved in the initial stages of AD, despite the lack of early clinical manifestations. This might be ascribed to its ability to plasticly reorganize itself via alternative circuits, even recruiting additional cortical relays in the sensorimotor system [Ferreri et al., 2003], due its natural distributed network with multiple representations of the motor maps [Sanes and Donoghue, 2000]. This ability seems to be supported by cortical hyperexcitability that is a well-defined neurophysiological feature of AD, also evident in the early stages of the disease [Di Lazzaro et al., 2002, 2004; Ferreri et al., 2003] and probably related to its severity and progression [Ferreri et al., 2011a; Khedr et al., 2011]. This has been indirectly evaluated by several transcranial magnetic stimulation (TMS) studies along the last twenty years [Cantone et al., 2014; Guerra et al., 2011; Rossini et al., 2013]. TMS observations have in fact demonstrated that motor cortex excitability is directly correlated with motor cortex plasticity in elderly subjects and demented patients [Guerra et al., 2015]. Moreover, fMRI and PET studies have established that recruitment of extra neural resources and neural hyperactivation in task-positive brain regions may allow elderly subjects to maintain normal cognition despite Aβ deposition [Elman et al., 2014; Oh and Jagust, 2013]. These mechanisms represent compensatory rather than aberrant phenomena [Elman et al., 2014].

Within this theoretical frame, the purpose of the present study was to further investigate the neurophysiological hallmarks of motor cortex functionality in AD. To follow our aim we used an emerging neurophysiological approach to record direct electroencephalographic (EEG) responses to the TMS of a given scalp site with millisecond resolution. Combining TMS with EEG allows for the non-invasive and simultaneous study of cortical excitability and time-solved effective connectivity directly at the cortical level and within the same time-window [Ilmoniemi et al., 1997]. This is not affected by various confounding effects (e.g., the functional state of peripheral strictures, such as the spinal cord or the neuromuscular unit, or attentional/cognitive individual bias). A network of neuronal connections is indeed engaged when TMS-

Abbreviations

AD	Alzheimer's disease
EEG	Electroencephalography
EOG	Electro-oculogram
EP	Evoked potentials
ERP	Event related potential
FDI	First dorsal interosseus muscle
GMFP	Global-mean field power
MEP	Motor evoked potential
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
RMT	Resting motor threshold
SAI	Short afferent inhibition
SLF	Superior longitudinal fasciculus
TEP	TMS-evoked EEG-potential
TMS	Transcranial magnetic stimulation

evoked activation extends from a stimulation site to other parts of the brain and the summation of synaptic potentials produces deflections of alternating polarity in scalp EEG signals. The signals start at a few milliseconds and last about 300 ms after the stimulus, first in the form of rapid oscillations and then as lower-frequency waves [Ilmoniemiet al., 1997]. The amplitude, latency, and scalp topography of single pulse TMS-evoked EEG responses after M1 stimulation have been clearly described in healthy people [Ferreri et al., 2011b, ; Komssi et al., 2004; Massimini, 2005; Paus et al., 2001; Van Der Werf and Paus, 2006] and are thought to depend on the stimulation intensity and functional state of the stimulated cortex [Massimini et al., 2005; Ferreri et al., 2014a]. They thus reflect directly and timely the excitability and effective connectivity of the stimulated cortex [for review see Ferreri and Rossini, 2013].

MATERIALS AND METHODS

Subjects

Twelve AD patients (five male, seven female; age range 72.4 ± 5.9 years; education: 8 ± 3.5 years; MMSE 20.8 ± 2.7) were recruited shortly after they were first diagnosed with dementia. All of the patients met the current diagnostic criteria [McKhann et al., 2011]. They received a score of at least 18 on the mini-mental state examination (MMSE),and their symptoms manifested themselves less than two years before they were enrolled. Moreover, they were all free of relevant behavioral disturbances. The patients underwent neuropsychological testing, including the Mental Deterioration Battery, the MMSE, a functional evaluation (Instrumental Activity of Daily Living scale), and evaluation of affective symptoms. They also underwent brain magnetic resonance imaging (MRI) and laboratory screening to rule out other causes of dementia. No clinical evidence of motor disturbances was found in any of the patients after detailed neurological evaluation. None of the patients were taking acetylcholinesterase inhibitors or other drugs known to influence corticospinal excitability during TMS, nor had they suffered from epilepsy. Further exclusion criteria, in line with international safety standards for TMS, were metallic prosthesis or fragments in the cranial and thoracic districts, tinnitus, previous retinal detachment and brain hemorrhage [Rossini et al., 2015].

The age-matched control group consisted of 12 healthy volunteers (six male, six female; age range 68.6 ± 7.1 years; education: 9.1 ± 4.5 years; MMSE 28.8 ± 1.2).

Both patients and subjects were right-handed (handedness score 0.70), as evaluated by the Handedness Questionnaire [Salmaso and Longoni, 1985] and were instructed to abstain from caffeine and alcohol the day before the experimental session.

Patients, caregivers and control subjects provided informed consent, and the study was approved by the local ethical committee.

Transcranial Magnetic Stimulation

Single pulse TMS (monophasic pulse configuration; Magstim Company Limited, Whitland, UK) of the left M1 was performed during a multi-channel EEG recording by means of a standard figure-of-eight double 70 mm coil oriented to elicit a posterolateral-anteromedial current flow in the brain. The virtual cathode of the coil was placed over the "hot spot" of the hand area of the left M1, which was defined as the point from which stimuli at the minimal excitability threshold of TMS triggered motor evoked potentials (MEPs) of maximal amplitude and minimal latency in the target hand muscle: the first dorsal interosseus muscle (FDI). Then, the resting motor threshold (RMT) was identified according to international guidelines as the stimulator output able to elicit reproducible MEPs (at least 50 μV in amplitude) in about 50% of 10–20 consecutive stimuli [Rossini et al., 2015]. The coordinates of the head, the EEG electrodes and the coil were determined and transferred to the same coordinate system with MRI template scans through a system for stereotaxic neuronavigation (SofTaxic Navigator System, EMS Italy). In this way TMS was continuously targeted to the located hot spot on the left M1. Each subject underwent a 1-h session consisting of about 100 TMS trials. The intertrial interval, which lasted 6-8 s, avoided habituation with repeated stimulation [Rossini et al., 2015]. The TMS was given supra-threshold with an intensity of 120% of the RMT. Amplitudes of MEPs-recorded bilaterally from the FDI muscle by means of Ag/AgCl-coated electrodes filled with conductive jelly in a belly/tendon montage- were measured between two major and stable peaks of opposite polarity. Latencies of the MEPs were measured at the maximum positive peak. Skin/electrode resistances were below10 KOhms.

EEG recordings

A piece of TMS-compatible EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) -that does not require pinning the preamplifier output to a constant level during TMS- was used, allowing for continuous data recording without saturation of the EEG signals [Ferreri et al., 2011b]. The EEG activity was continuously acquired from 32 scalp sites using electrodes positioned according to the 10–10 International System. Additional electrodes were used as ground and as reference. The ground electrode was positioned in Oz for maximal distance from the stimulating coil. The linked mastoid served as the reference for all electrodes. The click associated with the coil's discharge propagates through air and bone and can elicit an auditory N1–P2 complex at latencies of 100–200 ms [Massimini et al., 2005]. To mask coil-generated clicks a white noise -obtained from the waveform of the TMS click digitized and processed to produce a continuous audio signal with its specific time-varying frequencies [Massimini et al., 2005]- was continuously

delivered to the subject through earphones. We adjusted the masking volume until the subjects reported that the TMS click was not audible (always below 90 dB). To ensure wakefulness throughout the recording sessions, subjects were required to keep their eyes open and to fixate on a target over the opposite wall. The signals recorded were bandpass filtered at 0.1–1000 Hz and digitized at a sampling rate of5 kHz. In order to minimize overheating of the electrodes by the stimulating coil, TMS-compatible Ag/AgCl-coated electrodes were used. Skin/electrode impedance was kept below 5 kOhms. Horizontal and vertical eye movements were detected by recording the electro-oculogram (EOG). The voltage between two electrodes located to the left and right of the external canthi recorded horizontal eye movements. The voltage between reference electrodes and electrodes located beneath the right eye recorded vertical eye movements and blinks.

DATA ANALYSIS AND STATISTICS

Computer scientist engineers (L.V., F.V., S.P., D.P) and a biostatistician (P.P) with expertise in TMS and EEG analysis performed the data processing and statistical analysis. Data analysis was conducted with MATLAB 2008b version 7.7 (MathWorks, Natick, Mass.) and the public license toolbox EEGLAB [Delorme and Makeig, 2004]. All the EEG and EMG signals were split into segments (epochs), lasting 3s that included 1s of pre-stimulus and were baseline corrected (100 ms pre-stimulus). All epochs showing TMS-EEG evoked activity contaminated by extreme values were automatically rejected [Delorme and Makeig, 2004]: epochs exceeding ± 120 μV in the 20ms-1s window after stimulus or ± 100 μV in the 100ms-1s time window after stimulus were marked for rejection. Similarly, epochs showing EMG evoked activities contaminated by extreme values were rejected too: epochs exceeding ± 200 μV in the 100 ms-1s time-window after stimulus were marked for rejection. Rejection results were visually inspected and manually confirmed by an expert (F.F). After epochs selection, EEG signals were bandpass (2–80 Hz) and notch (50 Hz) filtered, down sampled from 2,500 Hz to 500 Hz and average referenced. Eventually, data were averaged for each subject in order to extract the subjects' ERPs; individual averages were then used for further analysis.

Time domain was the main aspect investigated. First, the global-mean field power (GMFP) -a measure of global brain activation calculated as the root mean-squared value of the EEG signal across all electrodes [Lehmann and Skrandies, 1980]- was calculated and a t-test was performed to evaluate significant differences between the examined groups (Alzheimer's and Controls). Next, for a first topographical assessment, we integrated in maps the EEG activity of each channel using three time-windows chosen upon the visual inspection of the GMFP activity, 6–22 ms, 24–90 ms, and 92–190 ms. We performed an ANOVA analysis (hierarchical model II type) with two factors (groups and channels) in order to assess differences in global excitability.

Then, a semi-automatic amplitude/latency measurement of each TMS-evoked EEG-potential (TEP)- identified at the vertex by a visual inspection of the TMS-evoked responses [Ferreri et al. 2011b,]- and MEP was carried out. For this measurement only TEPs visually recognizable after 20 ms from the TMS onset were considered. This value is greater than the usually assumed boundary value of 5ms [Ferreri et al., 2011a,b; Veniero et al., 2009].

Considering the data collected from both groups, two ANOVA analyses (hierarchical model II type) with two factors (groups and channels) were performed for each peak. The first ANOVA was performed on raw potential values with the aim of detecting global general differences among groups. When this difference was present, we then performed a second ANOVA analysis (hierarchical model II type) with two factors (groups and channels) on average-corrected potentials to determine whether the global difference was coupled with a spatial modulation in the excitability and, in this case, Bonferroni corrected marginal means were extracted and discussed. An unpaired t-test (Bonferroni corrected) was used for the study of the latencies. These procedures allowed us to evaluate whether the peaks were being modulated by groups' behavior. Only results indicating a statistically significant between-groups main effect were reported.

Finally, upon visual inspection, we found that an additional component was present at around 80 ms in the GMFP of the AD patients. Therefore, we conducted an additional analysis on this peak that was statistically contrasted, after a semi-automatic amplitude/latency measurement, against the 60 peak and the 100 peak by means of two ANOVA analyses (hierarchical model II type) with two factors: the peaks (two levels: 60 and 80 or 80 and 100) and the channels (32 levels), with dependent variable TMS–EEG amplitudes each. The 80 peak of AD patients was then contrasted with the nominal values of the Control group at the same latency to fully characterize the wave.

Cortical Sources Analysis of the ERPs

Current densities for the TEPs components that revealed more significant differences between AD and Control groups were estimated using sLORETA [standardized low resolution brain electromagnetic tomography; Pascual-Marqui et al., 2002] in Curry software (v 6.0.2, CompumedicsNeuroscan Ltd., Charlotte, NC), for illustrative purposes. Basically, sLORETA gives a single linear solution to the inverse problem of localization of brain function based on scalp recordings and produces images of standardized current density with no localization bias [Pascual-Marqui et al., 2002]. 3D T1-weighted images (Philips Achieva 3T, Philips Medical System, The Netherlands) were used to create a realistic head model as volume conductor. A

three-compartment boundary element model (BEM 7/9/10mm) and standard conductivity values were used (0.33 S/m for the brain fluid, 0.0042 S/m for skull, and 0.33 S/m for skin). The regularization parameter was automatically determined by the $\chi 2$ criterion method implemented in Curry. The analysis was performed with the ERP data obtained at the time points that showed local maxims in the GMFP (i.e., AD: 34, 42, 64, 82, 110 and 188 ms post-stimulus, and Control: 28, 44, 60, 92 and 200 ms post-stimulus).

The sLORETA method is a properly standardized discrete, linear, minimum norm, inverse solution method that solves the problem to compute the three-dimensional cortical distribution of the electric neuronal source activity from the EEG measurements which are recorded on the head surface [Vecchio et al., 2013]. Several independent research groups have repeatedly demonstrated that LORETA solutions are able to faithfully model cortical responses to sensorimotor events [Carretié et al., 2004]. However, it should be stressed that sLORETA has a spatial resolution (centimeters) lower than that of positron emission tomography and fMRI imaging (millimeters). The so-called sLORETA solutions consisted of voxel current density values that were able to predict ERP voltage at scalp electrodes. The sLORETA solutions predicting scalp ERPs were regularized to estimate distributed rather than punctual EEG source activity [Pascual-Marqui et al., 2002]. It should be remarked that the head template of the originals sLORETA package cannot account for differences in the individual cortical envelope as typically done in the analysis of functional magnetic resonance imaging (i.e., normalization, coregistering, smoothing).

RESULTS

Resting Motor Threshold and MEPs

The RMT was $57.9 \pm 7.4\%$ for Controls and $53.6 \pm 2.8\%$ for AD. The average MEP amplitude and 95% confidence interval were 417 ± 197 μV for Controls and 992 ± 673 μV for AD. According to an unpaired t-test, no statistical difference between the groups was seen in the RMT and MEPs amplitude.

TMS-Evoked EEG Responses

As already well described [lmoniemi et al., 1997; Massimini et al., 2005; Ferreri et al., 2011b,,b; for review see Ferreri and Rossini, 2013], the supra-threshold single pulse TMS of the left MI evoked in both the experimental groups EEG activity lasting up to 300 ms [for review Komssi and Kähkönen, 2006] and peaking on the GMFP at approximately 30, 44, 60, 100 and 180 ms post-TMS, as illustrated in Figures 1 and 2. Indeed in most AD patients (9 out of 12) an additional component was observed around 80 ms post stimulus (Fig. 2).

The GMFP analysis revealed an increase in amplitude between 20 and 150 ms post-stimulus in AD patients, which was maximal between 24 and 90 ms, as highlighted in the Figure 3B ($P < 0.01$). When the integrated cortical activity in the chosen time-windows (that is 6–22 ms, 24–90 ms and 92–190 ms, Fig. 3A) was calculated, the resulting maps confirmed a clear increase in the cortical excitability of AD patients only in the time-window 24–90 ms ($P < 0.05$). Significant differences were evident in the stimulated sensorimotor cortices (that is in the electrodes C3 P3 and CP1, Fig. 3A). Moreover, when looking at the individual latencies (the polarity according to that observed at the vertex: N7, P30, N44, P60, N100 and P180), the maps analysis showed a significant group difference in some waves, with AD showing a clear increase in the cortical excitability. More specifically, the analysis showed a significant group difference ($P < 0.05$) at the P30 wave, which peaked in FC1 in both groups. The statistical analysis of average-corrected potential showed a significant channel-group interaction with significant marginal means in C3, P3 and CP1 ($P < 0.05$ Bonferroni corrected, Fig. 4). The analysis did not show any significance at the N44 wave. Finally, the statistical analysis showed a significant group difference ($P < 0.05$) at the P60 wave, which peaked in C3 in both groups, while analysis of average-corrected potential showed a significant channel-group interaction with significant marginal means in CP5 ($P < 0.05$ Bonferroni corrected, Fig. 4). The latency analysis showed significant difference in the N100 ($P < 0.001$) with the CO anticipating AD (Fig. 3B).

Lastly in order to better describe the additional component seen in AD patients around 80 ms (Fig. 5) this was contrasted against the 60 and the 100 peaks. The first statistical analysis showed a significant global difference between the 60 and the 80 peaks (the 60 being globally stronger than the 80 ($P < 0.05$)) with a not significant channel-peak interaction, suggesting the same spatial topography for both waves. Conversely, the second statistical analysis between P80 and N100 showed a significant channel-peak interaction ($P < 0.05$), suggesting a different spatial topography. This topological difference was corroborated by the result of marginal means that highlighted a significant difference in the CP1 and in the C3 channels ($P < 0.05$ Bonferroni corrected). The statistical analysis of the 80 peak across groups (AD and CO, Fig. 5) showed a significant global difference among groups for the raw potentials, peaking the EEG activity in C3 in both groups. Meanwhile, the analysis of the average-corrected potentials showed a trend only in the group-channel interaction. A coarse view of the topology through four ROIs[1] and the application of an ANOVA analysis with two factors (groups and ROIs), showed a significant group-ROI

[1]We used the left-anterior (FC1-FC5-F3), right-anterior (FC2-FC5-F4), left-center-posterior (C3-P3-CP1-CP5), right-center-posterior (C4-P4-CP2-CP6) ROI, whose values have been obtained through the averaging of electrodes.

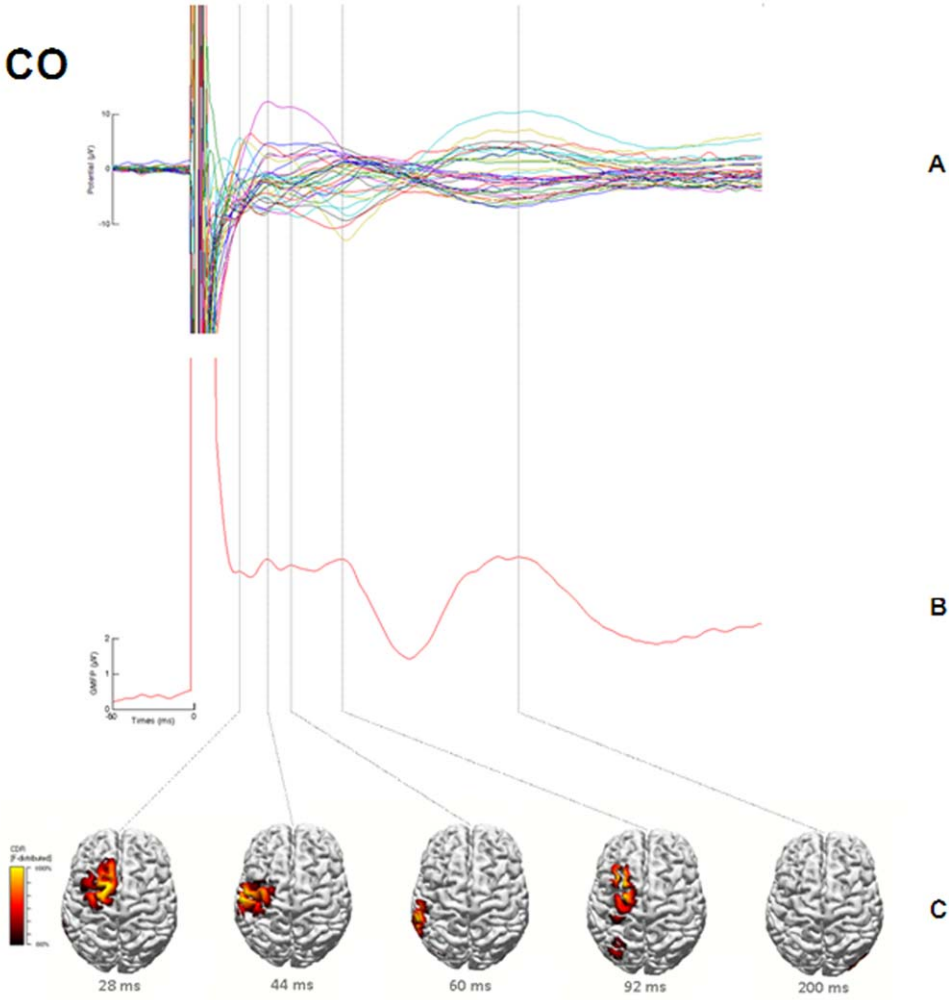

Figure 1.

Control subjects. (**A**) Averaged TMS evoked potentials recorded at all electrodes, superimposed in a butterfly diagram. (**B**) Global activation produced by TMS as measured by the GMFP. (**C**) Source localization of the activity occurring during each peak in the GMFP calculated using standardized low-resolution brain electromagnetic tomography (sLORETA) and plotted on the cortical surface. At each time point, the results were auto-scaled and thresholded at 80% to highlight maximum current sources. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

interaction, with a significant hyperexitability of AD groups in the center-left-posterior area. Then we evaluated the specificity and sensitivity of this component in AD patients, computing the amount of activations in CP1 and P3 with respect to C3. Considering as positive a patient showing an activation greater than or equal the 65% measured in C3, we found a value of sensitivity equal to 75% and a value of specificity equal to 75%.

Sources Computed by sLORETA

The Figures 1C and 2C show a grand average ($N = 12$ AD and 12 Control) of the sLORETA source solutions of the activity occurring during each peak of the GMFP, presented for illustrative purpose. The visual inspection of activation showed differences between AD and Control groups in response to magnetic stimulation.

It could be observed that after TMS healthy subjects showed current maxima (reflecting the center of neural activity) which shifted from the premotor cortex (around 30 ms), to the motor cortex (around 44 ms), to the somatosensory cortices (around 60 ms). Thus, in healthy brains the direct perturbation of the motor cortex is followed by spatially and temporally differentiated patterns of activation that appear to propagate along the anatomical connections of M1 with related cortical areas. In contrast, the

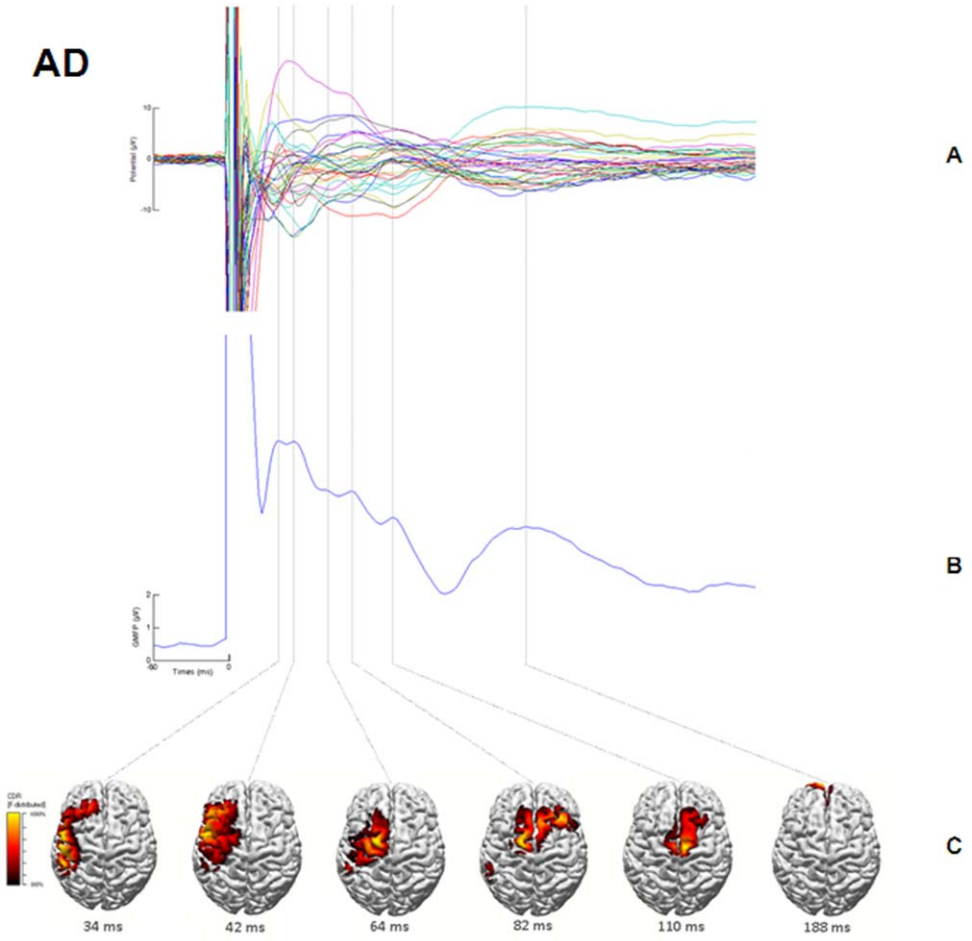

Figure 2.

Alzheimer's patients. (**A**) Averaged TMS evoked potentials recorded at all electrodes, superimposed in a butterfly diagram. (**B**) Global activation produced by TMS as measured by the GMFP. (**C**) Source localization of the activity occurring during each peak in the GMFP calculated using standardized low-resolution brain electromagnetic tomography (sLORETA) and plotted on the cortical surface. At each time point, the results were auto-scaled and thresholded at 80% to highlight maximum current sources. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cortical activation in AD patients remained broadly localized in the stimulated sensorimotor cortex for a longer period of time (since 30 to at least 60 ms). Thus, in AD, although the direct perturbation of the motor cortex elicits responses that are even stronger than those in healthy brains, these responses tend to remain in to the stimulated area, possibly reverberating and reinforcing locally.

DISCUSSION

Motor cortex hyperexcitability is a well-defined neurophysiological feature of AD patients, particularly those in the early disease stages [Di Lazzaro et al., 2002, 2004; Ferreri et al., 2003, 2011a; for review see Cantone et al., 2014]. This parameter is related to disease severity and progression [Ferreri et al., 2011a; Khedr et al., 2011]. However, to date, it has been extensively evaluated only via traditional TMS techniques, which provide indirect measures of motor cortex excitability alone. Here, by using the TMS-EEG co-registration approach, we have directly observed this phenomenon on the scalp (brain convexity) for the first time, extending it to the whole sensorimotor system. This hyperexcitability causes a stronger response to stimulation in a specific time window, possibly due to locally acting reinforcing circuits, while network activity and connectivity is reduced.

Several neuro-pathological studies in animals and humans have shown that -in contrast with traditional views- the motor cortex is involved in the early AD stages. Despite the lack of clinically evident motor deficits, which only appear in the later stages, the amount of Aβ

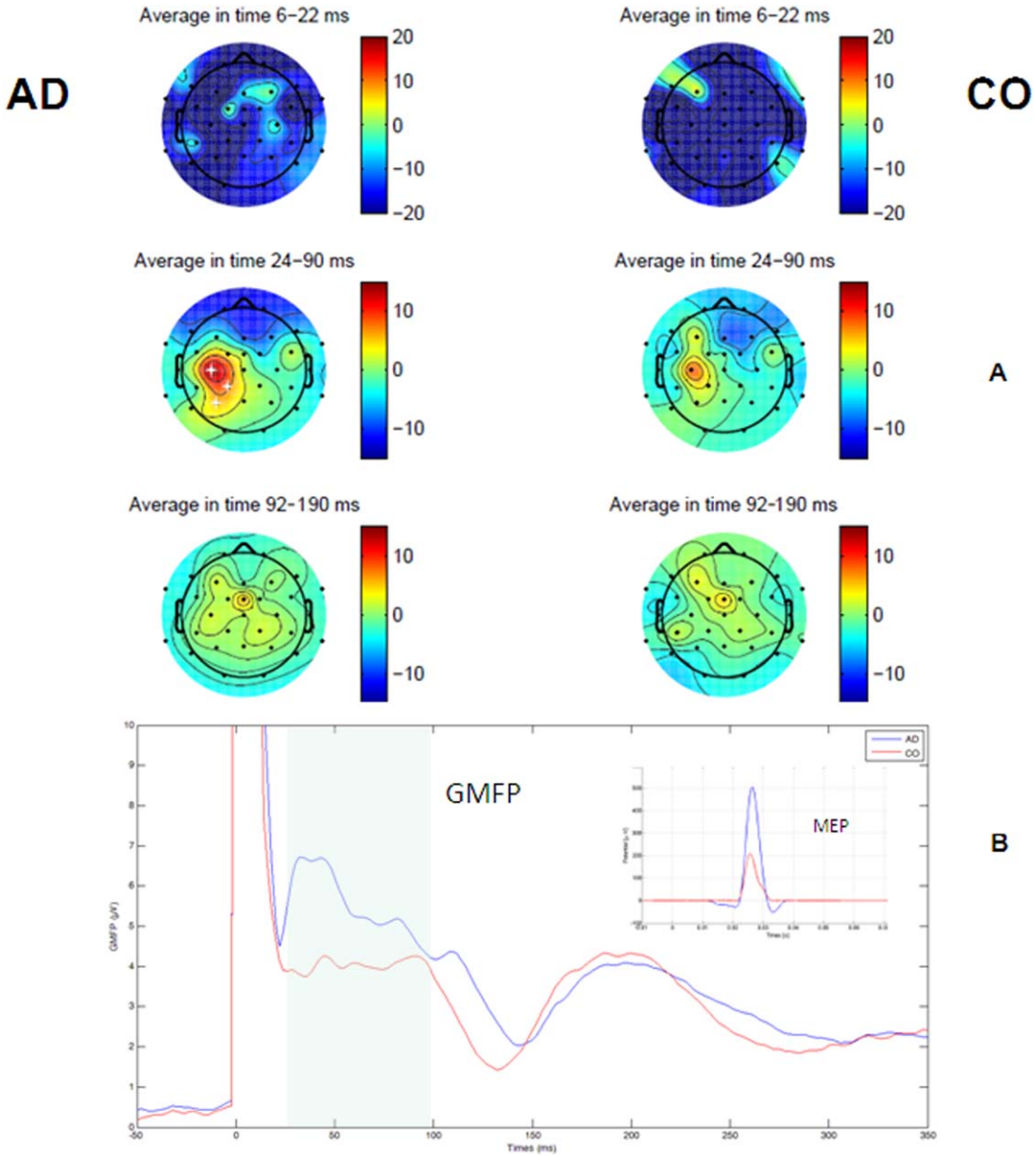

Figure 3.

Topographic distribution of the average integrated TMS evoked activity (A). Average integrated evoked response in CO subjects and AD patients for the time-windows 6-22, 24-90 and 92-190 ms after the TMS over the left M1; the white crosses indicate significant differences. (B) Average GMFP and MEP for each group superimposed for visual purpose. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

deposition in the motor cortex is comparable to that of other cortices generally considered more specific targets of the disease pathology, such as the enthorinal cortex and the hippocampus [Golaz et al., 1992; Mastrangelo and Bowers, 2008; Suvà et al., 1999; Yuan et al., 2013]. It is clearly emerging that the development of monomers-dimers-oligomers -leading to formation and deposition of beta-amyloid fragments and Aβ plaques- interferes with a variety of neural functions in the brain, including synaptic transmission. This finally induces aberrant local circuit properties [Koffie et al., 2009] and network disruptions [Arendt, 2009; He et al., 2009]. In the neocortex the particular effect of Aβ on the neural excitability has been the subject of a number of recent studies, most of them clearly

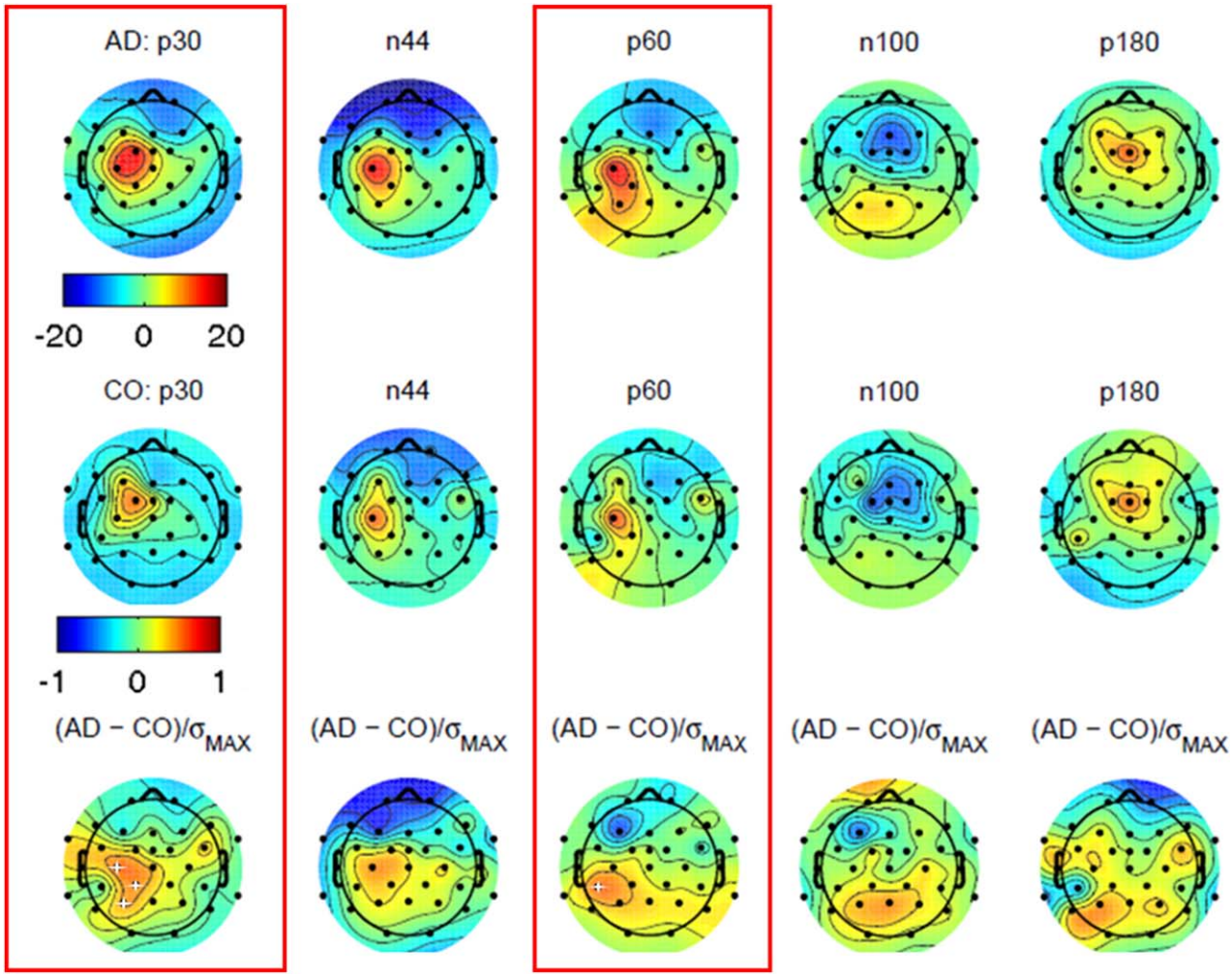

Figure 4.

Topographic distribution of the TMS evoked activity. Scalp distribution maps at 30, 44, 60,100 and 180ms after the TMS over the left M1 in CO subjects and AD patients and maps difference. The maps at 30 and 60ms are outlined and the white crosses indicate significant differences. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

showing patterns of Aβ induced hyperexcitability [Busche et al., 2008; Kellner et al., 2014; Palop and Mucke, 2010], in vitro and in the ongoing activity of transgenic mice brains [for review see Paula-Lima et al., 2013]. On the other hand, several mapping studies have revealed significant differences in the location and activity of various primary and secondary cortical motor centers in healthy volunteers [Teitti et al., 2008; Vaalto et al., 2011] and patients [Ferreri et al., 2003, 2014b]. Accordingly, the prevailing current idea regarding sensorimotor networks is that there are multiple presentations of functions and actions [Sanes and Donoghue, 2000]; this idea has relevant neurorehabilitation implications [Di Pino et al., 2014]. Based on the above-mentioned evidences [Vaalto et al., 2011], one could argue that supra-threshold stimuli on the motor cortex can also activate other representations outside the target muscle. If so, local mechanisms of pathological disinhibition could play a major role in the observed motor network rearrangement. In effect in previous studies on AD patients we have already proposed that the motor cortex hyperexcitability would be due to an imbalance between excitatory and inhibitory circuits probably induced by Aβ deposition [Ferreri et al., 2003, 2011a]. Then the crucial role of the glutamatergic over-activation has been demonstrated in AD recently. It stems from an imbalance between non-NMDA and NMDA neurotransmission in favor of the non-NMDA transmission in a complex mosaic that involves several neurotransmitter systems in a variety of brain areas [for review see Paula-Lima et al., 2013]. Moreover, it is well known that one of the

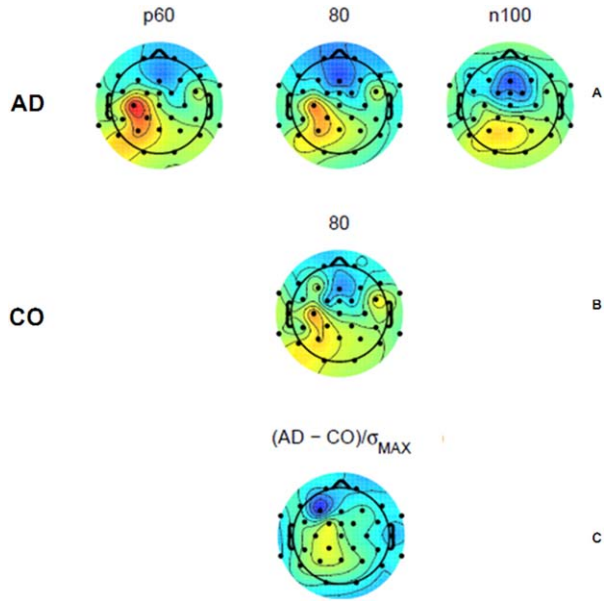

Figure 5.

Topographic distribution of the TMS evoked activity between 60 and 100 ms after the TMS in AD and statistical comparison at 80 ms with CO. (**A**) Scalp distribution maps at 60, 80 and 100 ms after the TMS over the left M1 in AD group. The overlapped topographical distribution between the P60 and the wave at 80 ms is evident. (**B**) Scalp distribution map at 80 ms after the TMS over the left M1 in CO group. (**C**) AD and CO groups map difference at 80 ms. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mechanisms of neuronal defence to compensate for excitotoxicity in response to glutamate over-activation is the GABA release [see review Paula-Lima et al., 2013]. Thus, also reduction of GABA mediated inhibition could be responsible for part of the cortical disinhibition. Finally several experimental observations suggest that also the well-known decrease in cortical Acetylcholine (ACh) levels may positively modulate the sensorimotor excitability in AD [Feig and Lipton, 1993, see also below].

The patho-physiological significance of these functional changes in the motor cortex excitability of AD patients is still poorly understood. Some evidence supports the notion that cortical networks hyperexcitability could promote cortical plasticity [Bella et al., 2011; Ferreri and Rossini, 2013; Polania et al, 2011], favoring the strengthening of existing synapses in a Hebbian way [for review see D'Amelio and Rossini, 2012], the recruitment of structurally existing but functionally silent synapses, and the driving of axonal outgrowth. These phenomena, finally modulating cortical connectivity, have been described both in healthy subjects [Elman et al., 2014] and in demented patients (as recently described by our group, Guerra et al., 2015).

Our results clearly show a strong cortical hyperexcitability in early AD, despite the lack of clinically evident motor manifestations. This would promote a rearrangement of motor cortex effective connectivity and can be then interpreted in the light of above. Actually, the visual inspection of grand average TEPs source modeling showed differences between AD and Control groups in response to the perturbation of the motor cortex, revealing in healthy brains spatially and temporally differentiated patterns of neural activation that appear to propagate back and forth along its anatomical connections. In contrast, the cortical activation in the AD brains, seems to be stronger than in the healthy brains and remains segregated to the stimulated area for longer, in a specific time-window between 60 and at least 80 ms post stimulus, possibly reverberating and becoming reinforced in sensorimotor local circuits. This higher local activity may be related to a reduced and/or disrupted network activity, which may compensate for a decreased functional sensorimotor connectivity. Then, by analysing EEG maps of TMS-evoked responses, we found the most significant differences between groups in a time-window from 24 to 90 ms post-stimulus, focussing around 30 ms post stimulus on the whole stimulated sensorimotor system and around 60–80 ms on the somatosensory cortices more selectively. Previous studies from our [Ferreri et al., 2011b] and other groups suggested a complex source structure for the wave P30 [Maki and Ilmoniemi, 2010; Van Der Werf et al., 2006]. Even though it plausibly not reflects directly the M1 activation induced by the stimulus it is considered one of the most informative TEP on the degree of excitation of sensorimotor network, including the prefrontal cortices, and the condition of intracortical GABAergic circuits [Ferreri, 2011b; Maki and Ilmoniemi, 2010]. Research suggests that the wave P30 reflects activity around the ipsilateral sensorimotor/premotor cortex border, in the superior wall of the ipsilateral cingulated gyrus or the supplementary motor area [Litvak et al., 2007] and might be an expression of the intrahemispheric spread of activation via cortico-cortical or a subcortical pathway with a diffused back-projection to the cortex [via thalamic nuclei and/or basal ganglia and/or Meynert's nucleus; Maki and Ilmoniemi, 2010], or both. However a contribution of the corpus callosum cannot be excluded. All these pathways are known to be functionally and structurally disrupted in AD [Hoeppner et al., 2012] with a clear involvement of the aforementioned balance between excitatory and inhibitory neurotransmission. On one hand the map analysis seems to suggest in AD patients rearranged cortico-cortical connectivity between the premotor, motor and parietal cortices that are anatomically interconnected through distinct white matter tracts forming the superior longitudinal fasciculus (SLF). SLF functionality has been indirectly demonstrated altered in AD by means of paired pulse TMS without clear ACh neurotransmission involvement [Bonnì et al., 2013]. On the other hand a similar rearranged effective connectivity would be also supported by the emerging early structural alteration of cortico-thalamic [Abuhassan et al., 2014] or

callosal interhemispheric circuits [Fjell and Walhovd, 2010] both of them variably insisting on excitatory and inhibitory intracortical interneurons.

Little is known about the source structure of the P60 peak. By studying the cortical correlate of short afferent inhibition (SAI) in healthy subjects we have posited that this wave could be the hallmark of the sensorimotor interaction [Ferreri et al., 2012], probing this transcortical route functionality and the corticocortical activation of GABAergic-mediated inhibition onto the corticospinal neurons probably modulated by cholinergic activation [Di Lazzaro et al., 2002, 2005]. A number of studies have examined the effects of cholinergic signalling on sensory processing in the sensorimotor coupling, where ACh release is both region and modality specific. In motor areas it is able to reduce intralaminar inhibition and promote intracolumnar inhibition while in somatosensory areas it improves the signal-to-noise ratio, enhancing stimulus discrimination [Xiang et al. 1998]. Here we suggest that this wave should be considered with particular interest as a probe of cortical cholinergic dysfunction in AD and is then worth of further experimental pursuit.

The adjunctive peak frequently seen in AD around 80 ms must be considered. Its cortical topography strongly resembles P60 even though on a lower level of global excitability. Between 60 and (at least) 80 ms in AD patients the signal doesn't spread. Rather, it seems to remain segregated in the sensorimotor area, possibly reverberating there. That is, it could be speculated a reverberant local circuit in the sensorimotor system, possibly supported by neural degeneration leading to disconnection and/or aberrant connectivity. This would fit with the presence of less networks activity/efficacy in AD, while the concomitant sensorimotor higher local activity may compensate for the decreased functional connectivity. These findings could be related to ACh depletion because cholinergic afferents to cerebral cortex can reorganize functional circuit structure as well regulate and control cortical plasticity and effective network connectivity by means of neurotrophic properties, while transient cholinergic deafferentation precipitates alterations in neuronal differentiation and synaptic connectivity [Bozzali et al., 2013]. The presence of an adjunctive peak also seems to be related to a strong increase of the latency of N100 in the AD population. It has been suggested that N100 could be used to investigate cortical activity in cognitive tasks, because it is sensitive to changes in attention, motor preparation and visual stimulus [Bender et al., 2005]. Latency increasing in evoked potential/event related potential (EP/ERP) has already been clearly described in AD (above all in the P300 response) and is possibly linked causally to the aforementioned disconnection phenomena (for a review see Vecchio and Maatta, 2011).

Few previous studies have used TMS-EEG recording to explore M1 excitability and connectivity in AD. Julkunen and coworkers, in particular, found a significantly reduced P30 amplitude in a small sample of AD patients that were assuming cholinesterase inhibitors [Julkunen et al., 2008, 2011]. The M1 disinhibition in AD has been shown to recover partly via the use of cholinesterase inhibitors [Di Lazzaro et al. 2005; Liepert et al., 2001], so contrasting experimental results could be ascribed to this aspect. Systematically studying the effect of cholinesterase inhibitors on the AD TEPs before and after acute and chronic drug intake could help us better understand this point.

CONCLUSION

There is a growing body of neuro-pathological evidence that -in contrast with traditional views- the motor cortex is actually already involved in the early AD stages, despite the lack of clinically evident motor deficits that only appear in the later stages [Golaz et al., 1992; Mastrangelo and Bowers, 2008; Suvà et al., 1999; Yuan et al., 2013]. The reasons for this discrepancy are still matter of debate and have been preliminarily ascribed to the motor cortex's ability to plasticly reorganize itself via alternative circuits, even recruiting additional cortices in the sensorimotor system [Ferreri et al., 2003, 2011a; Suvà et al., 1999]. By using TMS–EEG co-registration, the present study has clearly demonstrated that the sensorimotor system is deeply rearranged in mild AD patients without motor symptoms. Experimental features include strong cortical hyperexcitability, possibly supporting plastic reorganization of cortical connectivity with the recruitment of additional neural sources, the activation of reverberant local circuits and their integration in the distributed network subtending sensorimotor functions. These capabilities of plastic rearrangement would be ensured by the particular organization of the sensorimotor system based on the distributed network with a replicated topographic organization of the same body part [Sanes and Donoghue, 2000]. If these changes play some role in substantially preserving the sensorimotor performance in mild AD patients, they need to be confirmed by further studies. So far, however, they could be interpreted as a compensatory mechanism allowing for the preservation of sensorimotor programming and execution over a long period of time in spite of disease progression. The emerging possibility to externally and affordably modulate cortical excitability in healthy and pathological brains [Basso et al., 2006; Di Pino et al., 2014; Ferreri et al., 2006] along with a full comprehension of the above suggested compensatory mechanisms would open completely new and intriguing therapeutic perspectives in neurodegenerative dementing disorders.

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