Adaptability and reproducibility of a memory disruption rTMS protocol in the PharmaCog IMI European project

Pablo Martin-Trias[1], Laura Lanteaume[2], Elisabeth Solana[3], Catherine Cassé-Perrot[2], Sara Fernández-Cabello[1], Claudio Babiloni[4,5], Nicola Marzano[6], Carme Junqué[1], Paolo Maria Rossini[5,7], Joëlle Micallef[2], Romain Truillet[8], Estelle Charles[8], Elisabeth Jouve[8], Régis Bordet[9], Joan Santamaria[3,10,] , Jorge Jovicich[11], Simone Rossi[12], Alvaro Pascual-Leone[13,14], Olivier Blin[2], Jill Richardson[15], David Bartrés-Faz[1,3,*],

[1]Medical Psychology Unit. Department of Medicine. Faculty of Medicine and Health Sciences. University of Barcelona, Spain

[2]Department of Clinical Pharmacology, and CNRS UMR 7289, CIC-CPCET, Aix-Marseille University, Marseille, France

[3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

[4]Department of Physiology and Pharmacology, University of Rome "La Sapienza", Rome, Italy

[5]Department of Neuroscience, IRCCS San Raffaele Pisana, Rome, Italy

[6]IRCCS SDN, Naples, Italy

[7]Department of Geriatrics, Neuroscience & Orthopaedics, Catholic University, Policlinic Gemelli, Rome, Italy

[8]CIC-UPCET, Timone University Hospital, AP-HM, UMR CNRS Aix-Marseille University 6193, Marseille, France

[9]University of Lille, Inserm, CHU Lille, U1171 - Degenerative and Vascular Cognitive Disorders, Lille, France

[10]Sleep Unit, Neurology Department, Hospital Clinic, Barcelona, Spain

[11] Center for Mind/Brain Sciences (CIMEC), University of Trento, Trento, Italy

[12]Dipartimento di Scienze Mediche, Chirurgiche e Neuroscienze, Brain Investigation & Neuromodulation Lab. (Si-BIN Lab), University of Siena, Siena, Italy

[13]Berenson-Allen Center for Noninvasive Brain Stimulation and Division of Cognitive Neurology, Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, MA 02215, USA

[14]Institut Guttmann de Neurorehabilitacio, Universitat Autonoma de Barcelona, Spain

[15]Neurosciences Therapeutic Area, GlaxoSmithKline R&D, Stevenage, UK

* dbartres@ub.edu

Abstract

Transcranial Magnetic Stimulation (TMS) is a unique tool allowing the interference of cognitive processes, including the induction of transient memory impairment.

Within the context of a multicentric European project, we investigated the adaptability and reproducibility of a previously published TMS memory interference protocol in two centers using EEG and fMRI scenarios and further aimed to investigate the stability of the effects over two separated experimental sessions.

Sixty-eight young healthy men were included. Volunteers were instructed to remember visual pictures while receiving neuronavigated repetitive TMS (rTMS) trains (20 Hz, 900 ms) at picture encoding over the left dorsolateral prefrontal cortex (L-DLPFC) and the vertex area. Three experimental sessions were conducted, performing mixed ANOVA model analyses.

rTMS over the L-DLPFC lead to a significant interference during memory recognition. No differences were found between centers or between fMRI and EEG scenarios. Subjects with lower baseline memory performances were more permeable to TMS disruptive effects. No stability of memory interference could be demonstrated. Present data suggests sufficient robustness of the adapted rTMS protocol to be implemented in multicentric studies incorporating harmonized experimental procedures for direct effects comparability. However, intra-subject variability in responses to TMS may limit its applicability in crossover or longitudinal designs.

Introduction

Transcranial Magnetic Stimulation (TMS) is a non-invasive technique allowing painless stimulation of the brain, where brief pulses of current flowing through a coil of wire generate a time-varying magnetic field pulse. The rate of change of the magnetic field determines the induction of a secondary current in a conducting living tissue such as the cortical surface, and this secondary current may lead to the depolarization of the underlying populations of neurons[1]. Although a primary application of TMS technology is the study of the corticospinal motor system in Neurology and Neurophysiology[2], TMS and repetitive TMS (rTMS) have been widely used for many years in studies of Cognitive Neuroscience and Neuropsychology[3]. In this regard, and depending on the experimental conditions, TMS may act as a temporary enhancer of cognitive functions[4,5], or conversely, it may be used to transiently interfere with major cognitive domains, thereby gaining causal inferences on the role of the stimulated region in behaviour. As regards its applicability in cognitive neuroscience, a major breakthrough was the insight that TMS could be coupled with information from functional neuroimaging techniques[6]. Imaging information can be used to guide stimulation hence increasing the spatial precision of the brain area to be stimulated, as well as to investigate the effects induced on cerebral networks in terms of their functional reorganization in response to the magnetic pulses, and how this relates to a given behavioural outcome.

The present study incorporates the use of rTMS as one of the experimental arms within the framework of a European Commission Seventh Framework Programme (FP7/2007-2013, grant nº 115009), Innovative Medicine Initiative (IMI) 'PharmaCog' project (http://www.imi.europa.eu/content/pharma-

coq), which focuses on the early stages of drug development in Alzheimer's disease (AD)[7]. A series of 'cognitive challenge experiments' (including TMS but also sleep deprivation) were tested in young healthy human volunteers for their capacity to transiently disrupt cognitive tasks relevant for AD. Once the efficacy of the challenge models could be established, the reversibility of the induced dysfunction would be tested employing distinct pharmacological products. Hence, the overarching idea was that the approach would allow enabling experimental platforms that could be used to test for early indications of efficacy of newly developing compounds.

The aim of the present study was to test the adaptability and reproducibility of a TMS protocol previously published in the literature[2] to induce interference on memory processes. For this purpose, we tested its effects in two experimental centers, within two experimental scenarios and in two time points. To our knowledge, no data is available in the cognitive TMS literature testing for the replicability of cognitive effects in separate experimental sites. However, the potentiality of implementing non-invasive brain stimulation protocols in major clinical trials likely will depend on the capacity of developing standardized protocols that could be used in a multicentric approach[8]. A further relevant, - but yet untested - aspect for the potential incorporation of TMS in clinical trials requiring longitudinal or cross over designs, is to investigate the stability (i.e. test-retest reproducibility) of the observed findings, which therefore constitutes a further aim of the present study.

In this study we compare rTMS stimulation effects on memory recognition outcomes across different experimental conditions, namely Centre for Marseille (MRS) and Barcelona (BCN) and Modality for Electroencephalogram (EEG) and

functional Magnetic Resonance Imaging (fMRI) scenarios. However, the present manuscript focuses only on the behavioral findings (i.e. memory interference) and the study of the putative brain activity/connectivity changes underlying the observed effects will be analyzed in separate publications.

Results

Table 1 shows mean valid performances and standard deviations (SD) at encoding and recognition from Experimental Day 1 and 2 for the sample of participants included in the respective analyses of variance (ANOVA) For encoding accuracy, RT and FA analysis and results, see described results at supplementary material.

Main effects of TMS on memory performance (Day 1 vs Day 2 data)

As regards the impact of TMS on the main outcome, reflecting memory recognition performance, ANOVAs results showed a Condition main effect on Hits % ($F=11.95$, $p= 0.001$) and d' ($F=8.84$, $p=0.004$) was found. An interaction was also observed between Condition and Time on Hits % ($F=14.85$, $p<0.0005$) and d' ($F=9.43$, $p=0.003$). No significant effects were observed for Time (Hits %: $F=2.35$, $p=0.131$; d': $F=0.82$, $p=0.369$), neither for the factors Centre (Hits %: $F<0.01$, $p=0.947$; d': $F<0.01$, $p=0.987$) or Modality (Hits %: $F=0.29$, $P=0.589$; d': $F=0.11$, $p=0.744$), indicating that active rTMS over the Left dorsolateral prefrontal cortex (L-DLPFC) interfered with memory performance compared to

Vertex stimulation in a similar manner in both experimental sites and regardless of whether subjects were responding within the fMRI or EEG scenarios.

Post hoc analysis confirmed that memory performance was lower for L-DLPFC compared to Vertex only for Day 2 (Hits %: t=-5.09, p<0.0005; d': t=-4.67, p<0.0005) when active stimulation was applied, but not for Day 1 (Hits %: t=0.16 p<0.872; d': t=0.07, p<0.942, Fig. 3) where a sham coil was used as a placebo condition. These results remained when subanalyses considering each Modality and Centre were undertaken (Fig. 4).

Reproducibility of TMS effects (Day 1 vs Day 2 vs Day 3 data)

As described in the methods section we invited the subjects showing the greatest rTMS interferences at experimental Day 2 to attend for an equivalent experimental Day 3, conducted in average 15 days later. Comparing memory recognition performances on 21 individuals were data was collected at the three time points. A Condition main effect was found (Hits %: F= 13.09, p=0.002; d': F=7.68, p=0.013). Interaction between Time and Condition was also significant (Hits %: F=17.05, p<0.0005; d': F=14.46, p<0.0005). No main effect for Time was observed (Hits %: F=2.52, p=0.095; d': F=1.16, p=0.327). Post hoc analyses revealed that lower memory performance at retrieval under L-DLPFC condition compared to Vertex was observed only at Day 2 (Hits %: t=-10.05, p<0.0005; d': t=-9.20, p<0.0005), but not at Day 3 (Hits %: t=-0.77, p=0.448; d': t=-0.54, p=0.597), when active rTMS was also applied, or at Day 1 (Hits %: t=0.48, p=0.634; d': t=0.75, p=0.461; Fig. 4). Finally, similar to the main effects observed for the whole sample of individuals, we did not observe any significant

effects of Centre (Hits %: F=1.11, p=0.306; d': F=0.07, p=0.791) or Modality (Hits %: F=0.74, p=0.402; d': F=0.25, p=0.624).

Finally, in order to identify possible predictors of TMS interference, we compared baseline memory performance (i.e. at Day 1 where sham TMS was used) between the 26 individuals identified as sensitive to TMS (responders) and the 42 subjects who were not sensitive (non-responders) at Day 2. We found that *''responders'* exhibited lower memory performance at baseline both, for sham L-DLPFC and Vertex conditions (L-DLPFC Hits %: t=2.29, p=0.025; d': t=3.11, p=0.003; Vertex Hits %: t=2.53, p=0.014; d': t=3.59, p=0.001) compared with active L-DLPFC and Vertex respectively (see Fig. 5).

Discussion

The findings of the present study show independent replication of a TMS cognitive protocol, even if relevant experimental modifications were undertaken compared to initial published observations. Hence, overall our findings suggest that TMS holds potential to be incorporated in harmonized protocols of multicentric experimental or clinical trial investigations aiming to transiently induce episodic memory impairment in humans. However, we also observed important inter-individual variability in response to TMS and failed to demonstrate reproducibility of interference, even if we only selected individuals who initially demonstrated the TMS disruptive effects.

Over the years, accumulating evidence has unequivocally demonstrated the capacity of non-invasive brain stimulation to modulate cognition in humans ([5,9,10] for reviews). Despite this, there is a manifest lack of standardization of designs and procedures employed, which stands in sharp contrast with the widely established procedures to interrogate motor cortex functions using TMS[11–13]. Furthermore, in non-invasive brain stimulation cognitive studies, attempts to replicate the findings obtained using published protocols are very scarce. For example, while meta-analytical evidence indicates that single session of transcranial direct current stimulation (tDCS) modulates linguistic functions[14], recent data attempting to reproduce particular findings failed to replicate such observations[15].

To our knowledge, in the case of TMS, there are very few evidences of studies conducted by independent groups explicitly designed to replicate cognitive findings reported in the literature. Towards this end, we selected a memory interference protocol whose effects had been reported in several studies, in some cases using overlapping samples across studies[16]. Here we demonstrate that the adaptation of the core experimental procedures by two separate research groups confirmed the overall expected effects. Hence this data should be considered as a first step proving the potentiality of adapting cognitive TMS protocols in multicentric investigations using standardized protocols for later direct comparisons amongst experimental sites.

Despite these findings, in our study the distinctive average drop for memory recognition of L-DLPFC condition pictures compared with Sham-Vertex condition was around 7%. This is at variance with previously reported rates where performance drops ranged from 20%[17] to 24%[18]. Hence, it is likely that

we could detect a significant effect of TMS due to the larger sample included in our study as compared to the published ones (i.e. typically group sizes ranging from 13 to 28 individuals). For one of the Rossi's studies[16] there was a bigger sample (n=66) but divided in two subgroups, old and young subjects. Besides these aspects, the magnitude differences of the observed effects may be related to the fact that while keeping the core experimental procedures equivalent, we included some experimental adaptations to fulfill the PharmaCog project requirements and standards[7].

Deviations regarding the published protocol in the literature include performing an fMRI guided instead of pure anatomical landmark stimulation, as former studies indicated that greater sensitivity for behavioural effects may be achieved (e.g.[19]) using neuronavigation. This new approach implied that the stimulation target was placed posterior and laterally within the left middle frontal gyrus (L-MFG), compared to the average estimated F3 standard MNI localization[20] (Fig. 7). Past memory studies using TMS found that the particular target may play an important role on the behavioral cognitive outcomes observed. As an example, Blumenfeld et al.[21] found that stimulating left ventrolateral prefrontal cortex (L-VLPFC) before a verbal encoding task resulted in a subsequent memory disruption, whereas TMS over L-DLPFC facilitated memory recognition compared to a vertex condition.

A further relevant characteristic of our study sample is that it was entirely composed by men, whereas previous reports included both genders. Results, including both tDCS and TMS protocols, suggest that overall females may be

more responsive than men[22] for motor[23,24], visual[25], and some cognitive effects[26,27]. Accordingly, in an unpublished post-hoc analysis from the original series in the literature using the adapted protocol, it was found that most of the interferential effect was sustained by women (*Rossi, S. personal communication*). Nevertheless due to protocol restrictions, no women were included in the study.

Different average task difficulty could be another relevant variable accounting for the reduced magnitude of the interference effects compared to previous literature, as task difficulty likely interacts with TMS[28,29]. However our overall performance for Vertex (sham) stimulation was 79.04% (SD=12.49) of hits, which is comparable to the one reported in former investigations (i.e. hits values of 74%[17]; 76.2%[30]; 79%[31]; 72%[18]). Nevertheless, it should be noted that in our protocol, subjects were instructed with specific emphasis to intentionally remember the encoded stimulus, which was at variance compared to the original reports[16–18] where incidental memory encoding was undertaken. In the previous literature, when considering a semantic level of processing at encoding (e.g. category classification equivalent to indoor/outdoor used in the present model), no significant memory differences or brain activity patterns were found between intentional and incidental encoding[29,32–35]. On the other hand, a key variable that may result in performance differences is when the level of processing (i.e. deep/semantic vs shallow encoding) is specifically manipulated[29,33,36–38], which was not the case in the present report or the original studies. In the light of comparable overall memory performances between present task adaptation and the original reports in the literature[17,18],

our results appear to provide further evidence of the importance of 'level of processing' variable over 'incidental vs intentional encoding' to explain different memory outcomes.

Finally, our study allowed a direct comparison between stimulation of the Vertex area revealing no differences and no interference memory effects following sham and verum stimulation. Hence, these evidences are in the same direction of previous findings suggesting Vertex as a valid control condition when assessing memory function[17,21,29,39].

Despite the average overall effects observed, a relevant aspect of our study is that when considering individual subjects, only 40% of the participants showed a significant interference effect (i.e. their drop of memory performance comparing L-DLPFC to Vertex condition was at least -1SD). This is in agreement with previous results, as in López-Alonso study[40], where depending on the TMS and tDCS protocols, responsiveness values reached 40% in the expected direction, albeit other studies reported higher rates of responders, including 60% amongst healthy young subjects in intermitent theta burst stimulation (iTBS) protocols[41], 67%[42], 76%[43], and 78%[44] for Paired-associative stimulation (PAS) protocols and 75%[45,46] for TMS protocols.

Inter-individual variability has been increasingly recognized as an important effect explaining findings as well as discordances in motor studies[11,42]. Factors that could be contributing to inter-subject variability in the response to different

brain stimulation protocols, include methodological issues, such as coil orientation[47] as well as the previously alluded subject characteristics including age[22,42] and gender[23,25], but also time of the day[48], genetics[49], and baseline level of excitability[42,50,51] or short latency intracortical inhibition[40] (SICI).

As described before, the only variable that was predictive of the TMS response was baseline memory performance, in the sense that individuals with lower recognition memory performance at baseline were more permeable to stimulation effects. Similar evidences were found by another group concluding that high memory performers at baseline may implement more efficient compensatory processes making them more resistant to TMS interference effects than low memory performers[52].This observation is further reminiscent of former tDCS reports where baseline performance level resulted in greater positive[53,54] or negative[55] cognitive effects of stimulation. It is also in line with a recent transcranial alternate current stimulation (tACS) investigation in which tACS on the prefrontal cortex increased fluid intelligence capabilities in slow baseline performers, but not in baseline fast performers[56]. In our study greater interference in those with lower baseline memory performance may indicate reduced resilience or less optimal engagement of brain plasticity mechanisms to counteract the putative perturbation effects of TMS. Differences in cognitive reserve, which has been proposed to be a construct reflecting an index of brain plasticity and associated to greater efficiency of memory networks[57], could in principle be associated with the observed differences between subjects. However, years of education, a common proxy of reserve, and a variable previously shown to interact with brain stimulation effects (i.e.[58]), was

comparable between permeable and non-permeable individuals. In any case, the study of the mechanisms that may provide differential resilience to TMS interference would require neurophysiological data, such as comparisons between brain activity/connectivity patterns during the memory encoding task that served to guide TMS targeting, which was not available in our study.

One final relevant observation of our study is that we were unable to replicate the initial interference memory effect, when the same individuals were tested 15 days apart. Revising the existent literature on TMS effects reproducibility findings, a mixture of results appear, both highlighting negative findings[44–46,59–61] as well as different TMS protocols showing reproducible and reliable tools producing stable effects across sessions[41,62]. Importantly, none of these reports assessed reproducibility of cognitive effects within the same individuals in distinct experimental sessions.

López-Alonso et al.[40] observed that 39%, 45% and 43% of subjects responded as expected to PAS25, AtDCS, and iTBS brain stimulation protocols, but only 12% of individuals responded to all protocols in the expected direction. In our study, we selected 21 individuals clearly showing a disruptive effect of TMS on memory performance to be retested 15 days apart but only 19% (n=4) of them were consistently interfered at both experimental days. Hence, and along with the previous observations of the literature, our data using cognitive outcomes reinforces the notion that a particular response at one experimental time point may not be predictive of the same effect at a latter testing when considering particular individuals.

In the previous literature, proposed variables accounting for intra-subject variability effects included a non-complete overlapping of stimulation site between sessions[63], fluctuations in subjects' attention within and between sessions[64,65], individual's' history of physical activity[66] or variations on the levels of the stress hormone cortisol[67,68]. In our report the two first possibilities seem unlikely to have contributed to our findings as TMS was applied using the same neuronavigated fMRI-based coordinates during the two experimental sessions, and since performance during the encoding processes (i.e. reflecting attentional demands) were also comparable at both time points(Day 2 and Day 3). However, it should be noted that due to the main objective of our work focusing on memory interference effects, we did not collect data at the third experimental session of individuals that were not responsive to TMS at the second experimental day. This aspect limits the possibility of obtaining a more complete analysis of the aspects associated with reliability of intra-subject stability beyond the study of reproducibility of effects across sessions at a group level.

In conclusion, our study revealed positive replication of an existing cognitive TMS protocol, despite its adaptation to specific experimental purposes. No Centre differences regarding TMS cognitive effects appeared supporting TMS multicentric applicability. . Further, novel findings suggest that enriching studies with individuals exhibiting low baseline memory performance may result in greater observable TMS interference effects and hence reduce interindividual variability. Methodologically, comparability of null effects when stimulating the Vertex condition using either a real or a sham TMS coil confirms the use of this location as a good control condition when visual memory is studied. Finally, non-reproducibility of TMS effects across different time-points in a subsample of

previously interfered subjects was observed, an issue that needs to be addressed in further TMS cognitive investigations.

Method

Memory interference task and experimental design

Our review of the literature[9] identified a procedure leading to several publications where the application of high frequency rTMS during visual memory encoding resulted in disruption of memory performance during a later recognition phase[17]. After some task adaptations (see supplementary material) we created three equivalent tasks to be used in a counterbalanced order across three experimental days as it is depicted in fig. 1.

At Screening Day, subjects were familiarized with a short version of the memory task-procedure. Subjects enrolled must met eligibility criteria based on inclusion/exclusion criteria detailed in supplementary material. During the second visit (experimental Day 1), volunteers performed a complete memory encoding-retrieval experiment under sham TMS. Hence, Day 1 served to obtain a baseline of performance for each individual under fictitious brain stimulation, and was also used for enrolling procedures, as individuals performing less than 60% of correctly recognized items were not included in the final sample. At the third day (experimental Day 2), selected subjects performed an equivalent version of the encoding-retrieval memory task under active TMS. Finally, fifteen days later, a subsample of subjects were invited to undergo an identical experimental day (experimental Day 3), to test for reproducibility of TMS effects.

During both experimental Days 2 and 3 (real or active TMS), individuals performed the recognition task either inside the MRI scanner or while bearing the EEG cap (see below groups distribution). The study protocol was approved by the French ethics committee "SUD MÉDITERRANÉE I" and French regulatory authority ANSM (Agence Nationale de Sécurité du Médicament) and by Spanish local committee "Comité Ético de Investigación Clínica de l'Hospital Clínic" (CEIC) from Barcelona and was in accordance with the Declaration of Helsinki. All volunteers were properly informed and gave signed consent. The study was registered in ClinicalTrials.gov, for Spain and French locations (Number Identifier: NCT01861639, registered on May 23, 2013). In this work we are reporting just a specific part of the whole clinical trial related to the behavioural outcome of rTMS interference.

Encoding task consisted in 6 blocks of 12 pictures each (50% indoor, 50% outdoor, see Fig. 2a). After a 30 minutes break, subjects performed recognition memory task. The task included the presentation of 48 new pictures and 24 old pictures from Vertex and L-DLPFC conditions (Fig. 2b). Recognition task was performed in the same experimental room as encoding, for experimental Day 1. For experimental days 2 and 3 fMRI or EEG data were additionally recorded while subjects were undertaking the memory recognition phase.

Sample

A total of 68 young, healthy individuals (mean age: 24; SD: 4) took part in the study but only 21 completed till experimental Day 3 (sample distribution across Modalities and Centers: fMRI group: 56, 44 from BCN; EEG group: 12, 6 from

BCN). All included subjects were male due to protocol restrictions (see supplementary material).

MRI guided TMS protocol

TMS was applied using a MagPro X 100 Stimulator (MagVenture A|S, Denmark) combined with an eXimia Navigated Brain Stimulation (Nexstim, Finland) device for BCN subsample and using a Magstim Stimulator (Magstim Company Limited, USA, CE certification) combined with neuronavigation system Brainsight 2.2 (Rogue Research Inc., Montreal, QC CAN) for MRS subsample. Resting Motor threshold (rMTH) was determined at each experimental session as described in the International Standard Guidelines[69]. High frequency (20Hz) 900ms TMS trains were then applied at 500 ms of picture presentation, as this timing of stimulation exerted the clearest effects on memory interference[18], at 90% intensity of the individual rMTH, during the encoding blocks, alternatively over one of two brain regions. Vertex site (Cz location according to the 10-20 electrode placement[70]) as a control area and L-DLPFC site, determined from a previous fMRI memory study briefly described in supplementary material, as L-DLPFC has been widely related to encoding processes[71,72]. The region corresponds to the intersection between Brodmann areas 9/46, the limit between L-MFG and left inferior frontal gyrus (L-IFG) mean peak activation voxel according to Montreal Neurological Institute (MNI) coordinates (x,y,z): -42,10,30 (see Fig.7). Neuronavigated stimulation with stereotactic registration was performed to ensure accuracy in localization and position of TMS coil.

Data analyses

Behavioural responses were evaluated for every experimental condition. At encoding, accuracy was defined as the percentage of correctly categorized items as indoor or outdoor. Different sample size was used because of corrupted data of 3 subjects (n=65). Mixed ANOVAs were performed. Condition (Vertex vs L-DLPFC) and Time (Day 1 vs Day 2) were entered as within-subjects factor and Centre (BCN vs MRS) as a between subjects factor. Modality (EEG vs fMRI) was not entered because there were no protocol differences at the encoding phase. The same analyses were performed for Day 3 but with 3 Time levels (Day 1, Day 2 and Day 3, n=19, Bonferroni correction was used for multiple comparisons, p values <0.017 were considered significant).

For recognition, as main measures of memory performance, we considered Hits (percentage), mean reaction time (RT, reflecting the time elapsed between the presentation of a picture and the subsequent recognition motor response, for each modality subsample), false alarms (FA, which is a false recognized item) and d' (d prime, which is a combined index of sensitivity to correctly recognize seen and unseen targets by using the hit and false-alarm rates[73]). Mixed ANOVA was performed to evaluate TMS effects on memory performance (Hits%, d', RT). Time (2 levels: Day 1 and Day 2) and Condition (2 levels: L-DLPFC and Vertex) as within-subject factors. Centre (2 levels: MRS and BCN) and Modality (2 levels: EEG and fMRI) were entered as between-subject factors. To test for stability of TMS effects, we applied the same statistical model, adding a level to Time factor: Day 1, Day 2 and Day 3 (n=21, Bonferroni correction was used for multiple comparisons, significant p values <0.017 were

considered). For FA performance, were a single score is obtained at each experimental day, we performed an identical ANOVA model but only with Time as a within-subjects variable. The Greenhouse-Geisser correction was used if necessary to correct for non-sphericity. All effects are reported as significant at $p<0.05$. ANOVA main effects and interactions were further assessed using post hoc t tests. Data management and analysis were performed using Statistical Package for the Social Sciences 17.0 (SPSS Inc.) software packages.

To classify subjects as being sensitive to rTMS interference effects or not, mean Vertex performances were considered as a benchmark and all L-DLPFC performances that were below 12.5% of the corresponding Vertex performance (i.e. corresponding to -1SD of the group distribution) were considered as significantly interfered. Therefore, subjects showing at least 1SD drop at L-DLPFC condition performance compared to Vertex, were considered responders and eligible to participate in Day 3.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Hallett, M. Transcranial magnetic stimulation and the human brain. *Nature* **406,** 147–150 (2000).

2. Rossini, P. M. & Rossi, S. Transcranial magnetic stimulation: Diagnostic, therapeutic, and research potential. *Neurology* **68,** 484–488 (2007).

3. Walsh, V. & Pascual-Leone, A. *Transcranial magnetic stimulation : a neurochronometrics of mind*. (MIT Press, 2003).

4. Guse, B., Falkai, P. & Wobrock, T. Cognitive effects of high-frequency repetitive transcranial magnetic stimulation: a systematic review. *J. Neural Transm.* **117,** 105–122 (2010).

5. Bartrés-Faz, D. & Vidal-Piñeiro, D. Noninvasive brain stimulation for the study of memory enhancement in aging. *Eur. Psychol.* **21,** 41–54 (2016).

6. Paus, T. Imaging the brain before, during, and after transcranial magnetic stimulation. *Neuropsychologia* **37,** 219–24 (1999).

7. Galluzzi, S. *et al.* Clinical and biomarker profiling of prodromal Alzheimer's disease in workpackage 5 of the Innovative Medicines Initiative PharmaCog project: a 'European ADNI study'. *J. Intern. Med.* **279,** 576–591 (2016).

8. Floel, A. & Cohen, L. G. Recovery of function in humans: Cortical stimulation and pharmacological treatments after stroke. *Neurobiol. Dis.* **37,** 243–251 (2010).

9. Martin-Trias, P. *et al.* Translational Challenge Models in Support of Efficacy Studies: Neurobehavioral and Cognitive Changes Induced by Transcranial

Magnetic Stimulation in Healthy Volunteers. *CNS Neurol. Disord. Drug Targets* **15,** 802–815 (2016).

10. Luber, B. & Lisanby, S. H. Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* **85,** 961–970 (2014).

11. Hamada, M., Murase, N., Hasan, A., Balaratnam, M. & Rothwell, J. C. The role of interneuron networks in driving human motor cortical plasticity. *Cereb. Cortex* **23,** 1593–1605 (2013).

12. Vallence, A. M. *et al.* Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. *Neuroscience* **304,** 266–278 (2015).

13. Lin, T. *et al.* Effects of Theta Burst Stimulation on Suprahyoid Motor Cortex Excitability in Healthy Subjects. *Brain Stimul.* **10,** 91–98 (2017).

14. Price, A. R., McAdams, H., Grossman, M. & Hamilton, R. H. A meta-analysis of transcranial direct current stimulation studies examining the reliability of effects on language measures. *Brain Stimul.* **8,** 1093–1100 (2015).

15. Vannorsdall, T. D. *et al.* Reproducibility of tDCS Results in a Randomized Trial. *Cogn. Behav. Neurol.* **29,** 11–17 (2016).

16. Rossi, S. *et al.* Age-Related Functional Changes of Prefrontal Cortex in Long-Term Memory: A Repetitive Transcranial Magnetic Stimulation Study. *J. Neurosci.* **24,** 7939–7944 (2004).

17. Rossi, S. *et al.* Prefontal cortex in long-term memory : an ‘ interference ’

approach. **4,** (2001).

18. Rossi, S. *et al.* Temporal dynamics of memory trace formation in the human prefrontal cortex. *Cereb. Cortex* **21,** 368–373 (2011).

19. Sack, A. T. *et al.* Optimizing Functional Accuracy of TMS in Cognitive Studies: A Comparison of Methods. *J. Cogn. Neurosci.* **21,** 207–221 (2009).

20. Keeser, D. *et al.* Prefrontal Transcranial Direct Current Stimulation Changes Connectivity of Resting-State Networks during fMRI. *J. Neurosci.* **31,** 15284–15293 (2011).

21. Blumenfeld, R. S., Lee, T. G. & D'Esposito, M. The effects of lateral prefrontal transcranial magnetic stimulation on item memory encoding. *Neuropsychologia* **53,** 197–202 (2014).

22. Todd, G., Kimber, T. E., Ridding, M. C. & Semmler, J. G. Reduced motor cortex plasticity following inhibitory rTMS in older adults. *Clin. Neurophysiol.* **121,** 441–447 (2010).

23. Chaieb, L., Antal, A. & Paulus, W. Gender-specific modulation of short-term neuroplasticity in the visual cortex induced by transcranial direct current stimulation. *Vis. Neurosci.* **25,** 77–81 (2008).

24. De Gennaro, L. *et al.* Callosal effects of transcranial magnetic stimulation (TMS): The influence of gender and stimulus parameters. *Neurosci. Res.* **48,** 129–137 (2004).

25. Kuo, M.-F., Paulus, W. & Nitsche, M. a. Sex differences in cortical neuroplasticity

in humans. *Neuroreport* **17,** 1703–1707 (2006).

26. Fumagalli, M. *et al.* Brain switches utilitarian behavior: Does gender make the difference? *PLoS One* **5,** (2010).

27. Knops, A., Nuerk, H. C., Sparing, R., Foltys, H. & Willmes, K. On the functional role of human parietal cortex in number processing: How gender mediates the impact of a 'virtual lesion' induced by rTMS. *Neuropsychologia* **44,** 2270–2283 (2006).

28. Beck, S. & Hallett, M. Surround inhibition is modulated by task difficulty. *Clin. Neurophysiol.* **121,** 98–103 (2010).

29. Innocenti, I. *et al.* Event-related rTMS at encoding affects differently deep and shallow memory traces. *Neuroimage* **53,** 325–330 (2010).

30. Floel, A. *et al.* Prefrontal Cortex Asymmetry for Memory Encoding of Words and Abstract Shapes. *Cereb. Cortex* **14,** 404–409 (2004).

31. Rossi, S. *et al.* Prefrontal and parietal cortex in human episodic memory: An interference study by repetitive transcranial magnetic stimulation. *Eur. J. Neurosci.* **23,** 793–800 (2006).

32. Becker, N., Kalpouzos, G., Persson, J., Laukka, E. J. & Brehmer, Y. Differential Effects of Encoding Instructions on Brain Activity Patterns of Item and Associative Memory. *J. Cogn. Neurosci.* **29,** 545–559 (2017).

33. Craik, F. I. M. & Tulving, E. Depth of processing and the retention of words in episodic memory. *J. Exp. Psychol. Gen.* **104,** 268–294 (1975).

34. Stark, C. E. L. & Okado, Y. Making memories without trying: medial temporal lobe activity associated with incidental memory formation during recognition. *J. Neurosci.* **23,** 6748–53 (2003).

35. Buckner, R. L., Wheeler, M. E. & Sheridan, M. A. Encoding processes during retrieval tasks. *J. Cogn. Neurosci.* **13,** 406–15 (2001).

36. Vidal-Piñeiro, D. *et al.* Task-dependent Activity and Connectivity Predict Episodic Memory Network-based Responses to Brain Stimulation in Healthy Aging. *Brain Stimul.* **7,** 287–296 (2014).

37. Otten, L. J., Henson, R. N. & Rugg, M. D. Depth of processing effects on neural correlates of memory encoding: relationship between findings from across- and within-task comparisons. *Brain* **124,** 399–412 (2001).

38. Otten, L. J. & Rugg, M. D. Task-dependency of the neural correlates of episodic encoding as measured by fMRI. *Cereb. Cortex* **11,** 1150–60 (2001).

39. Galli, G., Feurra, M., Pavone, E. F., Sirota, M. & Rossi, S. Dynamic changes in prefrontal cortex involvement during verbal episodic memory formation. *Biol. Psychol.* (2017). doi:10.1016/j.biopsycho.2017.02.008

40. 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.* **7,** 372–380 (2014).

41. Hinder, M. R. *et al.* Inter- and intra-individual variability following intermittent theta burst stimulation: Implications for rehabilitation and recovery. *Brain Stimul.* **7,** 365–371 (2014).

42. Müller-Dahlhaus, J. F. M., Orekhov, Y., Liu, Y. & Ziemann, U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp. Brain Res.* **187,** 467–475 (2008).

43. Stefan, K., Wycislo, M. & Classen, J. Modulation of Associative Human Motor Cortical Plasticity by Attention. *J. Neurophysiol.* **92,** 66–72 (2004).

44. Fratello, F. *et al.* Modulation of corticospinal excitability by paired associative stimulation: Reproducibility of effects and intraindividual reliability. *Clin. Neurophysiol.* **117,** 2667–2674 (2006).

45. Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H. & Pascual-Leone, A. Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. *Exp. brain Res.* **133,** 425–30 (2000).

46. Maeda, F., Gangitano, M., Thall, M. & Pascual-Leone, A. Inter- and intra-individual variability of paired-pulse curves with transcranial magnetic stimulation (TMS). *Clin. Neurophysiol.* **113,** 376–82 (2002).

47. Talelli, P., Cheeran, B. J., Teo, J. T. H. & Rothwell, J. C. Pattern-specific role of the current orientation used to deliver Theta Burst Stimulation. *Clin. Neurophysiol.* **118,** 1815–1823 (2007).

48. Sale, M. V, Ridding, M. C. & Nordstrom, M. A. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp. brain Res.* **181,** 615–26 (2007).

49. Cheeran, B. *et al.* A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to

rTMS. *J. Physiol.* **586,** 5717–5725 (2008).

50. Hordacre, B. *et al.* Variability in neural excitability and plasticity induction in the human cortex: A brain stimulation study. *Brain Stimul.* **10,** 588–595 (2017).

51. Silvanto, J., Muggleton, N. & Walsh, V. State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* **12,** 447–454 (2008).

52. Manenti, R., Cotelli, M., Robertson, I. H. & Miniussi, C. Transcranial brain stimulation studies of episodic memory in young adults, elderly adults and individuals with memory dysfunction: A review. *Brain Stimul.* **5,** 103–109 (2012).

53. Tseng, P. *et al.* Unleashing potential: transcranial direct current stimulation over the right posterior parietal cortex improves change detection in low-performing individuals. *J Neurosci* **32,** 10554–10561 (2012).

54. Hsu, T. Y., Tseng, P., Liang, W. K., Cheng, S. K. & Juan, C. H. Transcranial direct current stimulation over right posterior parietal cortex changes prestimulus alpha oscillation in visual short-term memory task. *Neuroimage* **98,** 306–313 (2014).

55. Learmonth, G., Thut, G., Benwell, C. S. Y. & Harvey, M. The implications of state-dependent tDCS effects in aging: Behavioural response is determined by baseline performance. *Neuropsychologia* **74,** 108–119 (2015).

56. Santarnecchi, E. *et al.* Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. *Cortex* **75,** 33–43 (2016).

57. Bartrés-Faz, D. & Arenaza-Urquijo, E. M. Structural and functional imaging

correlates of cognitive and brain reserve hypotheses in healthy and pathological aging. *Brain Topogr.* **24,** 340–357 (2011).

58. Berryhill, M. E. & Jones, K. T. tDCS selectively improves working memory in older adults with more education. *Neurosci. Lett.* **521,** 148–151 (2012).

59. Boroojerdi, B. *et al.* Reproducibility of intracortical inhibition and facilitation using the paired-pulse paradigm. *Muscle Nerve* **23,** 1594–7 (2000).

60. Humm, A. M., Z'Graggen, W. J., von Hornstein, N. E., Magistris, M. R. & Rösler, K. M. Assessment of central motor conduction to intrinsic hand muscles using the triple stimulation technique: normal values and repeatability. *Clin. Neurophysiol.* **115,** 2558–66 (2004).

61. Schilberg, L., Schuhmann, T. & Sack, A. T. Interindividual Variability and Intraindividual Reliability of Intermittent Theta Burst Stimulation-induced Neuroplasticity Mechanisms in the Healthy Brain. *J. Cogn. Neurosci.* **29,** 1022–1032 (2017).

62. Vernet, M. *et al.* Reproducibility of the effects of theta burst stimulation on motor cortical plasticity in healthy participants. *Clin. Neurophysiol.* **125,** 320–326 (2014).

63. Carroll, T. J., Riek, S. & Carson, R. G. Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *J. Neurosci. Methods* **112,** 193–202 (2001).

64. Ellaway, P. H. *et al.* Variability in the amplitude of skeletal muscle responses to magnetic stimulation of the motor cortex in man. *Electroencephalogr. Clin.*

Neurophysiol. **109,** 104–13 (1998).

65. Funase, K., Miles, T. S. & Gooden, B. R. Trial-to-trial fluctuations in H-reflexes and motor evoked potentials in human wrist flexor. *Neurosci. Lett.* **271,** 25–8 (1999).

66. Cirillo, J., Lavender, A. P., Ridding, M. C. & Semmler, J. G. Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J. Physiol.* **587,** 5831–5842 (2009).

67. Sale, M. V., Ridding, M. C. & Nordstrom, M. A. Cortisol Inhibits Neuroplasticity Induction in Human Motor Cortex. *J. Neurosci.* **28,** 8285–8293 (2008).

68. Clow, A. *et al.* Day differences in the cortisol awakening response predict day differences in synaptic plasticity in the brain. *Stress* **17,** 219–223 (2014).

69. Rossini, P. M. *et al.* Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. *Clin. Neurophysiol.* **126,** 1071–1107 (2015).

70. Jasper, H. H. Report of the committee on methods of clinical examination in electroencephalography. *Electroencephalogr. Clin. Neurophysiol. Suppl.* **10,** 370–375 (1958).

71. Kapur, S. *et al.* Neuroanatomical correlates of encoding in episodic memory: levels of processing effect. *Proc. Natl. Acad. Sci. U. S. A.* **91,** 2008–11 (1994).

72. Tulving, E., Markowitsch, H. J., Kapur, S., Habib, R. & Houle, S. Novelty encoding

networks in the human brain: positron emission tomography data. *Neuroreport* **5,** 2525–8 (1994).

73. Stanislaw, H. & Todorov, N. Calculation of signal detection theory measures. *Behav. Res. Methods. Instrum. Comput.* **31,** 137–49 (1999).

Aknowledgements

This work was supported by the European Commission Seventh Framework Programme (FP7/2007-2013), Innovative Medicine Initiative (IMI) 'PharmaCog' project. P. Martin-Trias hold a fellowship from the Ministerio de Educación y Ciencia, Spain (12135072325-79).

We thank the volunteers who participated in this study

Author contributions

CB, JM, SR, DBF, PMR, RT developed the research questions and designed the study. CJ, APL, OB, RB provided detailed comments and assistance on the initials versions. JR, JM, LL, DBF were in charge of the trial management. PMT, LL, CCP, ES programmed the experiments. CB, NM, JS, SR, adapted the protocol to EEG conditions. PMT, ES, SFC, JJ, DBF adapted the protocol to MRI conditions. PMT, LL, ES, CCP, SFC assisted with recruitment of participants and data acquisition. EC, EJ were in charge of data entry and verification. PMT, SFC, CB, NM, RT, JJ data analyses. PMT, CCP, SR, APL, OB, DBF writing of the manuscript. JJ, DBF, PMT, CB, SR, APL interpretation of the results and discussion. All the authors read and approved the final manuscript.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

Figure legends:

FIG1: Figure 1. Main Design. a) General experimental design with four main timepoints and requirements subjects needed to fulfill to get through the whole study. SCR refers to Screening. L-DLPFC vs VERTEX DROP ≥ 1SD* refers to L-DLPFC performance drop of at least 1 standard deviation (12.5%) from Vertex performance. b) Memory protocol performed for each experimental day (Day 1, Day 2 and Day 3). MTH refers to Motor Threshold estimation. Drug test is composed by urine sample and a breath test.

FIG2: Figure 2. Memory task depiction. In the encoding part, **a**) each trial consisted of a fixation cross (variable timing), a red cross (warning 1 sec), a picture (1 sec), and a green cross (1 sec). Participants were asked to answer whether the picture was of an indoor or an outdoor scene by pressing the “z” or “m” keys on a standard computer keyboard respectively, after the appearance of the green cross. In the recognition part, b) each trial included a fixation cross, a red cross (1 sec), a picture (2 sec), and a green cross (1 sec). Participants were asked to answer if they had seen or not each picture by pressing the “z” or “m” keys on a standard computer keyboard respectively or in a magnetic resonance image (MRI) compatible keyboard where left button corresponded to “seen pictures” and right button to “unseen pictures”.

FIG3: Figure 3. L-DLPFC condition interference. Behavioural results for Experimental Day 1 and Day 2 in recognition memory task shown in mean Hits % and mean d'. Error bars shown on standard error of the mean (SEM).

FIG4: Figure 4. Interference across centers and modalities. Hits % consistent significant drop over L-DLPFC condition at Day 2 analyzed: over Modalities, a) fMRI group (* t=-4.16, p<0.005), b) EEG group (* t=-3.10, p=0.01); and across Centers, c) Barcelona group (* t=-3.91, p< 0.005), d) Marseille group (* t=-3.38, p=0.004). Error bars shown on SEM.

FIG5 : Figure 5. Interference reproducibility. TMS Behavioural results for Day 1, Day 2 and Day 3 in recognition memory task shown in Mean D' and Mean Hits % in a subsample (n=21). All $p < 0.05$. Error bars shown on SEM.

FIG6 : Figure 6. Baseline difference. Baseline memory performance differences ($p< 0.05$) between non-responders and responders subgroups for both L-DLPFC and Vertex conditions compared with Day 1 performance. Error bars shown on SEM.

FIG7 : Figure 7. TMS over L-DLPFC. Left hemisphere sagital view. Green circle represents L-DLPFC stimulation point at Montreal Neurological Institute (MNI) space coordinates (x,y,z): -42,10,30; and blue one, F3 location at MNI space coordinates (x,y,z): -34,26,44.

Table legend:

TABLE1 : Table 1. Performance summary. Mean performance ± SD of the total valid sample included in the Mixed ANOVA TMS study. For RT (Reaction Time),

data is shown in Modality subgroups in milliseconds (ms); RT EEG for recognition task reaction times under Electroencephalogram recording; RT fMRI when functional magnetic resonance was being acquired. FA for false recognized items (False Alarm) and d' as an index of memory sensitivity of correctly recognized seen pictures and correctly rejected unseen pictures.* L-DLPFC significant differences compared to vertex condition (Hits%: t=-5.07, p<0.0005; d': t=-4.67, p<0.0005).

Figure 1.

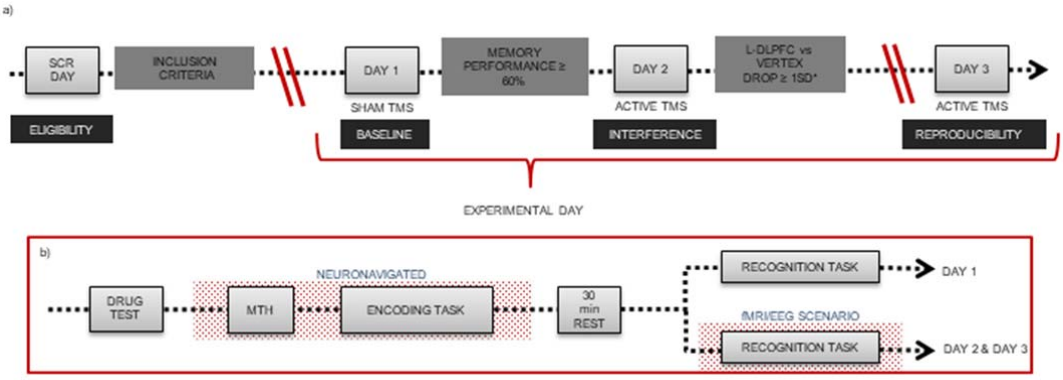

Figure 2.

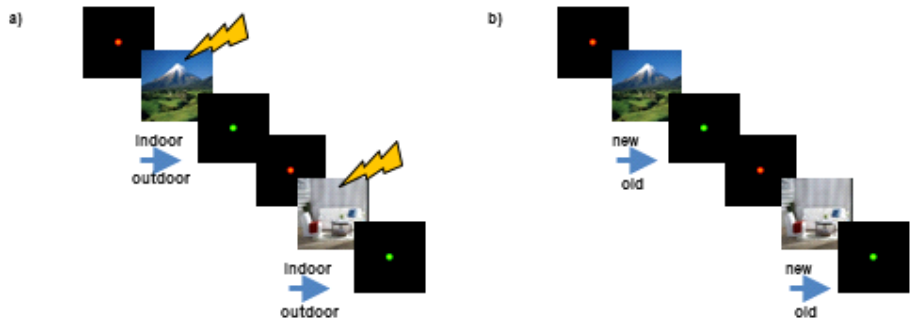

Figure 3.

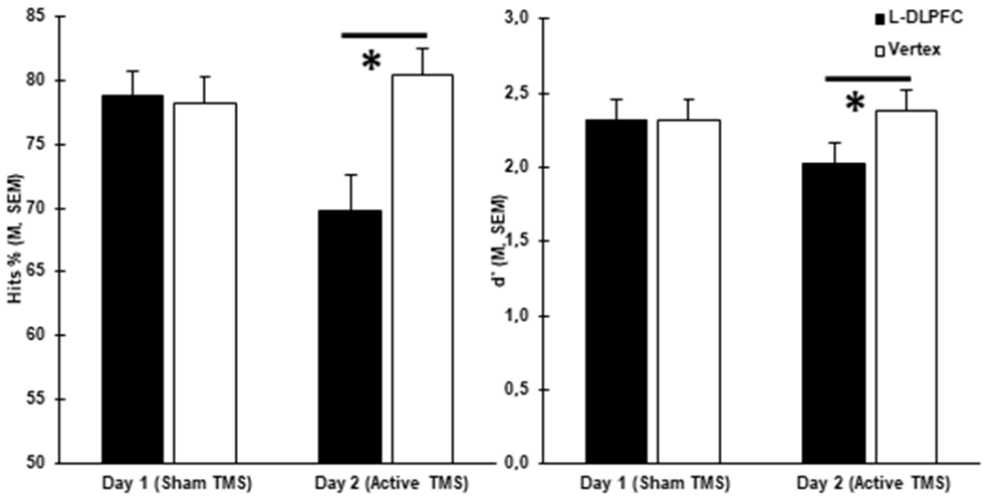

Figure 4.

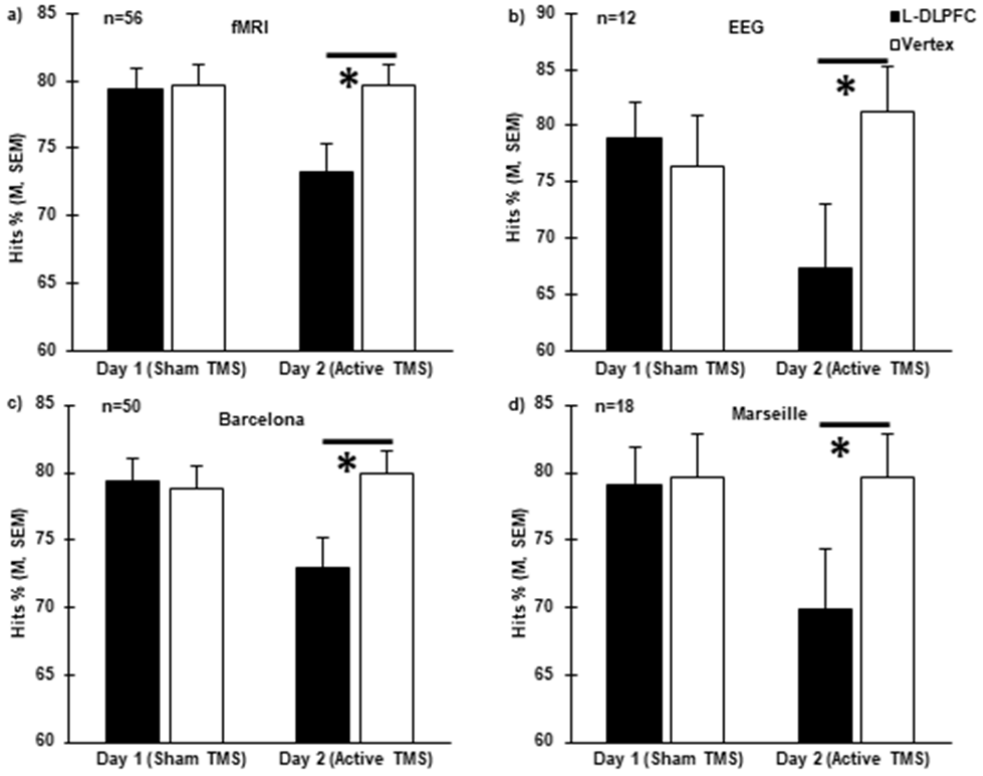
a)
n=56
fMRI
Hits % (M. SEM)
Day 1 (Sham TMS)
Day 2 (Active TMS)
b)
n=12
EEG
L-DLPFC
Vertex
c)
n=50
Barcelona
d)
n=18
Marseille

Figure 5.

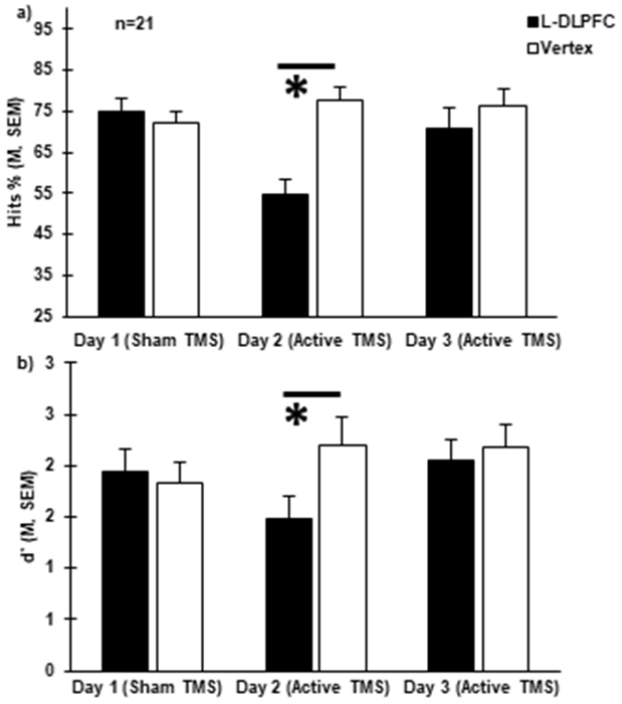

Figure 6.

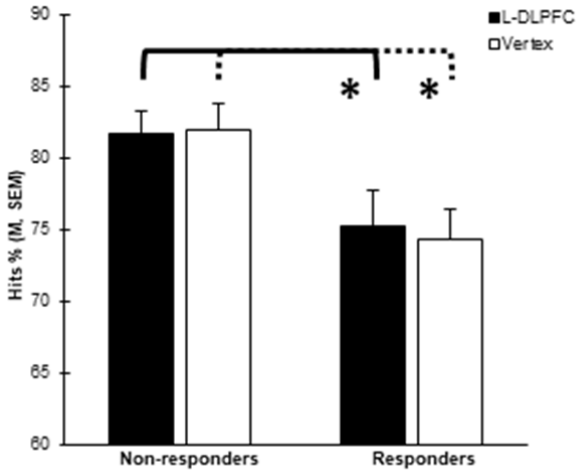

Figure 7.

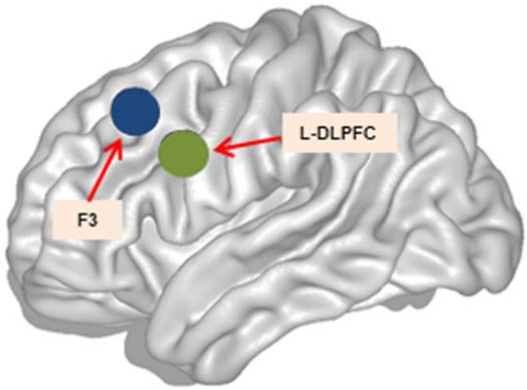

Table 1.

mean ± SD	n	Experimental Day 1 (sham TMS)		Experimental Day 2 (active TMS)	
		Vertex	L-DLPFC	Vertex	L-DLPFC
ENCODING					
Accuracy (%)	65	97.1±3.5	97 ±4	97.3±4	96.1±5
RT fMRI (ms)	54	717±247	776±523	794±232	817±238
RT EEG (ms)	11	948±356	923±28	833±312	903±344
RECOGNITION					
Hits (%)	68	79±12.5	79.3±12	79.9±12	72.2±16 *
FA (%)	68	10.4±10		11.5±11	
d'	68	2.3±1	2.3±1	2.3±1	2±1 *
RT fMRI (ms)	56	1234±311	1248±348	1192±355	1214±365
RT EEG (ms)	12	723±168	719±209	669±198	716±190