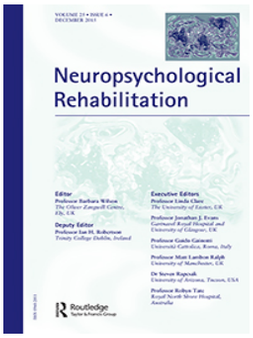

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Transcranial direct current stimulation and cognitive training in the rehabilitation of Alzheimer disease: A case study

Barbara Penolazzi[1], Susanna Bergamaschi[2], Massimiliano Pastore[3], Daniele Villani[2], Giuseppe Sartori[1], and Sara Mondini[1,2,4]

[1]Department of General Psychology, University of Padua, Italy
[2]Casa di Cura "Figlie di San Camillo", Cremona, Italy
[3]Department of Developmental and Social Psychology, University of Padua, Italy
[4]Human Inspired Technologies Research Centre-HIT, Padua, Italy

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In the present study we tested the cognitive effects of transcranial direct current stimulation (tDCS) in a case of probable Alzheimer disease (AD). The patient (male, 60 years, mild AD) underwent two cycles of treatments, separated by 2 months. In the first cycle, active stimulation (10 sessions, 2 mA for 20 min; anode over the left dorsolateral prefrontal cortex) was followed by computerised tasks (CTs) specifically chosen to engage the most impaired cognitive processes in the patient (tDCS+CT condition). In the second cycle, which was structured as the first, CTs were administered after placebo stimulation (sham+CT condition). Effects on cognitive performance were evaluated not only by the CTs, but also by neuropsychological tests assessing global cognitive functioning. Statistical analyses revealed that whereas the tDCS+CT condition had few effects on the CTs, it induced a stability of the patient's global cognitive functioning lasting approximately 3 months, which was not achieved when the patient underwent sham+CT condition. Therefore, the synergetic use of tDCS and CTs appeared to slow down the cognitive decline of our patient.

Correspondence should be addressed to Barbara Penolazzi, Department of General Psychology, University of Padua, via Venezia, 8 - 35131 Padova, Italy. E-mail: barbara.penolazzi@unipd.it

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This preliminary result, although in need of further confirmation, suggests the potentiality of tDCS as an adjuvant tool for cognitive rehabilitation in AD.

Keywords: tDCS; Alzheimer disease; Dementia; Brain stimulation; Cognitive rehabilitation; Neuroplasticity

INTRODUCTION

Investigating alternative, or complementary, and low-cost therapeutic treatments for Alzheimer disease (AD) is unquestionably a research priority given the extent of the disease and the social and economic consequences it brings about (Ballard et al., 2011). Along with pharmacological treatments, cognitive interventions have demonstrated some efficacy (see, for example, Bergamaschi, Arcara, Calza, Villani, & Mondini, 2013), and research is very active in attempting to understand the robustness of these results, by specifically testing the potential benefits derived through multi-approach treatments. Within this context, non-invasive brain stimulation could be a powerful tool for boosting the effects of cognitive treatments in AD (Boggio et al., 2011). Relatively few sessions of repetitive transcranial magnetic stimulation (rTMS), one of the most used non-invasive brain stimulation techniques, associated with cognitive training, have been shown to improve the performance in specific neuropsychological tests up to 2–3 months after the treatment (Bentwich et al., 2010; Rabey et al., 2013). However, data are not sufficient to establish evidence for rTMS therapeutic efficacy in this clinical context.

Non-invasive neuromodulation by transcranial direct current stimulation (tDCS) could be more promising than rTMS for AD patients in light of the many advantages it offers in comparison with magnetic stimulation (Nitsche et al., 2008): it shows no relevant side effects, it is less invasive, less expensive, simpler to use, more controllable, and potentially portable (thus possibly suitable for home treatments in the future). Many recent studies have confirmed the efficacy of tDCS in altering, by facilitation or inhibition, several cognitive processes, both in healthy individuals and in psychiatric and neurological patients (Miniussi & Vallar, 2011; Nitsche et al., 2008; Nitsche & Paulus, 2011; Vallar & Bolognini, 2011). However, the effects of tDCS on AD patients' cognitive functions are still poorly investigated, and very few studies addressing this issue have been reported in the literature. Two of them (Boggio et al., 2009; Ferrucci et al., 2008) have proved that one single session of anodal tDCS (on temporo-parietal, temporal, or prefrontal areas) is able to induce a short-term improvement in recognition memory of words and pictures. A third study (Boggio et al., 2012) has found that five consecutive daily sessions of anodal tDCS on the temporal cortex produced an improvement in picture recognition

memory lasting at least 1 month after the treatment. Finally, a recent study of Cotelli and coworkers (2014), targeted to investigate the effects of 10 sessions of anodal tDCS over the left prefrontal cortex, combined with memory training on face–name associations, failed to observe a significant effect of active tDCS on memory performance in AD. This brief survey of the literature shows that evidence to assess the therapeutic value of tDCS in AD is still insufficient. However, given the limited effectiveness of the currently available interventions to treat AD, research on the potentialities and limitations of this non-invasive neuromodulation technique unquestionably deserves more attention. Further studies are needed, not only to find the optimal tDCS parameters of efficacy, but also to shed light on some unsolved critical questions like, for example, the relationships between tDCS and cognitive rehabilitation, and the time extension of the stimulation-induced effects.

In the present case study, an AD patient underwent 10 daily sessions (*treatment phase*) of stimulation, followed by computerised tasks (CTs). The latter were used as a kind of cognitive training to rehabilitate the most impaired processes in our patient. The term "cognitive training" is commonly used to indicate many different behavioural interventions for cognitive enhancement (from those using more structured methods to those using more unspecific and freely managed approaches). In the present context, we used this term to refer to the set of CTs (always the same in each session) aimed at stimulating both the cognitive processes which are generally defective in AD patients (i.e., mnestic functions), and the cognitive processes which were specifically impaired in our patient (i.e., executive functions). The use of cognitive tasks targeted to engage the most impaired cognitive processes was aimed at activating the neural networks subserving those processes, thus (possibly) producing the neuroplastic changes needed to support their enhancements. The association of this neuronal activation with that induced by the stimulation had, in turn, the goal of testing whether the conjunction of these two types of intervention could boost cognitive and behavioural improvements. In order to increase the number of measures necessary for data analyses in single-case studies, the same CTs (without stimulation) were administered for 10 daily sessions before the treatment phase (*pre-treatment phase*, serving as a baseline), and for 10 daily sessions after the treatment phase (*post-treatment phase*, serving as a follow-up). After approximately 2 months from the first cycle, the patient underwent a cycle of treatment identical to the former, except for the fact that active stimulation was replaced by sham. tDCS effects on cognitive processes have been evaluated not only by the CTs executed in each protocol phase, but also by neuropsychological tests assessing global cognitive functioning. These tests have been administered at different time-points of the protocol to detect possible transfer effects on cognitive tests different from the trained CTs. The first aim of the case study was to provide further data on the potential therapeutic

effects of tDCS on cognitive deficits in Alzheimer disease, in the short- and the medium-terms, by monitoring the patient for a 6-month period. The second research aim was to test whether tDCS, used in conjunction with tasks which specifically engaged the most impaired cognitive processes in our patient, could be more effective than the tasks alone in improving his cognitive functions.

METHODS

Case study

The patient, a 60-year-old, right-handed man, with a high educational level (18 years) was referred to the outpatient service *Unità di Valutazione Alzheimer* [Alzheimer Evaluation Unit] of the medical facility *Figlie di San Camillo* in Cremona (Italy) in October 2011, due to a progressive worsening of memory and attention problems, which started approximately 2 years before referral. The neuropsychological evaluation revealed a mildly altered global cognitive performance on the Mini Mental State Examination (Magni, Binetti, Bianchetti, Rozzini, & Trabucchi, 1996; adjusted score: 23.2/30), and in the *Esame Neuropsicologico Breve-2* [Brief Neuropsychological Examination-2, ENB-2] (Mondini, Mapelli, Vestri, Arcara, & Bisiacchi, 2011; 60/100, a score lower than the cut-off of 73/100), whereas cognitive reserve, assessed by the Cognitive Reserve Index questionnaire (Nucci, Mapelli, & Mondini, 2011), was high (i.e., average score: 100, patient's score: 125). The diagnosis pointed to a probable primary degenerative dementia of Alzheimer type, also supported by neuroimaging evidence (Magnetic Resonance Imaging and 18-fluorodeoxyglucose positron emission tomography (PET-FDG)), revealing mild cortical atrophy and hypometabolism in bilateral parietal (most prominent in the left hemisphere) and left fronto-temporal areas. After the diagnosis, he was treated with rivastigmine (4.6 mg/24 h) initially combined with psychological support and with cognitive training. At 7-month follow-up, the neuropsychological profile remained stable compared with the first evaluation, whereas the PET-FDG of the same period highlighted a substantial enhancement of hypometabolism. This picture, being typical of dementia patients with high cognitive reserve (Solé-Padullés et al., 2009), confirmed the degenerative nature of the patient's disease. For this reason, the rivastigmine dose was increased (9.5 mg/24 h). Given the patient's willingness to undergo all the available treatments, we proposed a tDCS protocol for him, which he enthusiastically accepted. Therefore, the patient, who met the inclusion criteria for participating in non-invasive brain stimulation studies, was enrolled in the present protocol nearly 12 months after his first clinic visit. He gave his written informed consent

to participate in the study, which was approved by the local ethics committee and followed the principles of the declaration of Helsinki.

Tasks and procedure

A single-blind, sham-controlled design was used. The patient underwent two structurally identical cycles of stimulation (Figure 1), each including three identical phases: (1) *Pre-treatment phase* (serving as a baseline), consisting of 10 daily sessions (in 2 weeks) in which the patient only performed the CTs; (2) *Treatment phase,* consisting of 10 daily sessions (in 2 weeks) in which the patient first underwent the stimulation and then performed the same CTs as the previous phase; (3) *Post-treatment phase* (serving as a follow-up), consisting of 10 daily sessions (in 2 weeks) in which the patient only performed the same CTs as the previous phases. In the first cycle (tDCS+CT condition), the stimulation (20 min), followed by the CTs (approximately 45 min), was real; in the second cycle (sham+CT condition) the stimulation was simulated.

The CTs were selected on the basis of both the relevant literature (e.g., recognition memory; Ferrucci et al., 2008; Boggio et al., 2009, 2012) and the specific cognitive deficits of the patient (i.e., working memory, executive functions). Ten alternative task versions (one for each daily session of each phase) were administered to avoid possible learning effects induced by repeated practice of the same stimuli (except for the Continuous Performance Task, which did not suffer from this confound). Tasks were administered in counterbalanced order within each protocol phase. Neuropsychological assessment was included at different time-points to detect possible transfer effects on general cognitive performance (Figure 1).

Computerised tasks

Word Recognition Task (WRT). The WRT was a computerised version of the paper-and-pencil WRT successfully used by Ferrucci et al. (2008) in AD

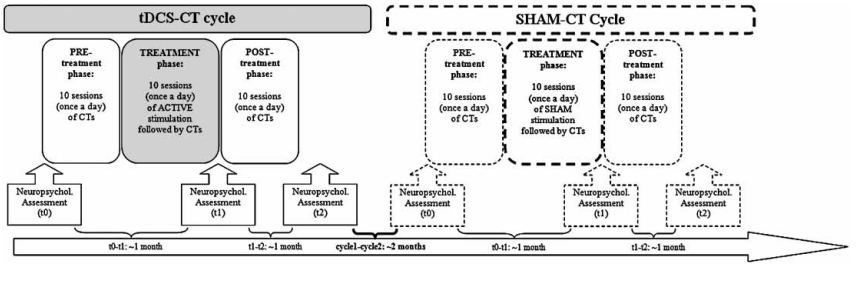

Figure 1. Timeline of the experimental protocol. CTs = computerised tasks.

patients. In detail, during a first phase (encoding), the patient had to learn 12 words (2000 ms, ISI: 1000 ms). In the second phase (recognition), administered 5 minutes after the previous phase (during which the patient performed an overt arithmetic calculation task), the previously displayed words were represented, randomly mixed with an equivalent number of new words. The patient was asked to judge if the words displayed in the recognition phase have been presented before. To avoid learning effects, 10 parallel lists were developed and randomly assigned to the 10 sessions of each protocol phase. The lists were matched for number of letters, familiarity, concreteness, imageability, and adult written word frequency according to the *LexVar* database (Barca, Burani, & Arduino, 2002). Within each list, old and new words were matched for the same variables as above. To avoid memory effects potentially lasting over a phase, we systematically inverted old and new words of each list from one phase to the next.

Verbal Working Memory Task (VWMT). In the VWMT, 60 pairs of five consonant strings were presented (S1: 5000 ms; fixation cross: 500 ms; S2: displayed until the response was given). Thirty pairs consisted of two identical strings, and 30 pairs consisted of two diverse strings in which the position of the changing letter was roughly equiprobable. Identical string pairs were presented interspersed with different string pairs. The patient was asked to judge if the two strings of each pair were identical. Ten parallel versions of this task were developed and randomly assigned to the 10 sessions of each phase.

Phonemic Fluency Task (PFT). A standard version of the PFT was used: the patient was given 1 minute to produce as many words as possible starting with a given consonant. Ten consonants were randomly assigned to the 10 sessions of each protocol phase.

Continuous Performance Task (CPT). A CPT modelled on Conners' task (Conners, Epstein, Angold, & Klaric, 2013), and included in the Psychology Experiment Building Language test battery (Mueller, 2010; http://pebl.sourceforge.net/), was used as a sustained attention task. The patient was required to respond by pressing the space bar to all letters randomly presented one at time, except for *X*-letter. The 360 total stimuli (90% *non-X*-stimuli, 10% *X*-stimuli) were displayed for 250 ms, with a variable inter-stimulus interval (1000, 2000, 4000 ms).

Neuropsychological assessment

To detect possible transfer effects on cognitive tests different from the trained CTs, in each treatment cycle, paper-and-pencil tests from the

battery ENB-2 (Mondini et al., 2011) were administered before the beginning of the pre-treatment phase (t0), immediately after the treatment phase (t1: 1 month after t0), and 2 weeks after the end of the post-treatment phase (t2: 1 month after t1; see Figure 1). Normative data of these tests are available for 1-month test–retest. Thus, by statistically comparing these data with test–retest measures of our patient (using the Modified Reliable Change Index-1, see Chelune, Naugle, Luders, Sedlak, & Awad, 1993; Collie, Darby, Falleti, Silbert, & Maruff, 2002), it was possible to establish whether any change in his performance could be considered a significant change, rather than a non-significant random fluctuation.

Transcranial direct current stimulation

Transcranial direct current was delivered through a battery-driven constant current stimulator (Eldith DC-Stimulator, NeuroConn GmbH, Germany), using a pair of surface saline-soaked sponge electrodes. In the active stimulation, a constant current of 2 mA was applied for 20 minutes (with 1 minute ramping up and down) in each of the 10 sessions of the treatment phase. The anode (5×7 cm^2; current density: 0.06 mA/cm^2) was positioned over the left dorsolateral prefrontal cortex (LDLPFC, F3 sensor of 10–20 EEG system) and the cathode (10×10 cm^2; current density: 0.02 mA/cm^2) over the right supra-orbital area. Repeated sessions of stimulation in potentially relevant areas in AD, such as temporal cortices, were found selectively to improve recognition memory, leaving other processes unchanged (see Boggio et al., 2012). Therefore, we targeted the LDLPFC based on data showing that its stimulation can often improve different types of cognitive processes, such as working memory, recognition memory, phonemic fluency, and executive functions (Boggio et al., 2009, Enriquez-Geppert, Huster, & Herrmann, 2013; Fregni et al., 2005; Iyer, Mattu, Grafman, Lomarev, Sato, & Wassermann, 2005), despite some evidence to the contrary in AD (see Cotelli et al., 2014). In the sham condition, the current was administered for 10 s at the beginning of the daily 20-minute stimulation period. During both active and placebo stimulations, the patient listened to relaxing music and performed the CTs when stimulation ended.

Data analysis

For the CTs, analyses were carried out in the R-environment (R Development Core Team, 2013). The many measures collected for each protocol phase allowed us to use mixed effect regressions, whose strength is the possibility of considering the whole structure of data as a combination of fixed and random effects, leading to enhanced statistical power (Pinheiro & Bates, 2000). This allowed us to overcome the main problem of single-case studies, that is the inadequateness of interpreting results and drawing

conclusions from data that are mostly analysed only qualitatively. For WRT, VWMT and CPT we analysed response accuracy and correct RTs. Response accuracy, which is a dichotomous variable, was analysed using GLMM (Generalised Linear Mixed Model; lme4 package; Bates & Sarkar, 2006) with logit link function (i.e., logistic regression). RTs, which were distributed according to the ex-Gaussian distribution (Luce, 1986; Van Zandt, 2000), were analysed using GAMLSS (Generalised Additive Model for Location Scale and Shape; GAMLSS package; Rigby & Stasinopoulos, 2005) with the ex-Gaussian link function. Ex-Gaussian distribution can be typified as the convolution of a normal and an exponential distribution, having three parameters: μ (the mean of the normal component), σ (the standard deviation of the normal component), and τ (the exponential component of the distribution). In these tasks, for both accuracy and parameters of the ex-Gaussian RT distribution, two fixed factors were considered in the analyses: *Treatment phase* (pre-treatment, treatment, post-treatment) and *Treatment type* (tDCS+CT, sham+CT); *Session* was entered in the analyses as a random factor. For the PFT, we analysed the number of produced words in each session, which is a count, using GLMM with log link function (i.e., Poisson regression; Fox, 1997, 2002). Also in this case *Treatment phase* and *Treatment type* were included in the analysis as fixed factors, whereas the *Letter* given in each session as a cue to perform the task was entered as a random factor. For all of the dependent variables, we performed a model-fit analysis, aimed at detecting the best-fitting model. Therefore, all the possible models (from the simplest, i.e., the null model, to the most complete model, i.e., the model with the interactive effect of the predictors) were built, then models were compared using the Bayesian Information Criterion (BIC, Schwarz, 1978) as fit index (the best-fitting model showing the smallest BIC value). For a given dependent variable, a model-fit analysis revealing that the best model was the null model has to be interpreted as a lack of significant effects of our predictors on that variable.

For the neuropsychological tests of ENB-2, in each cycle, the performance after the treatment phase (t1) was statistically contrasted with that in the pre-treatment phase (t0), and the performance after 1 month from t1 (t2) was compared with that in t1. These analyses, based on the comparison with the test–retest scorings of a normative sample (Mondini et al., 2011), allow one to establish whether a given test–retest change can be considered significant, rather than a random non-significant fluctuation due to inter-test variability or to practice effects (Chelune et al., 1993; Collie et al., 2002).

RESULTS

The active stimulation was well-tolerated by the patient and no side effects were reported.

Computerised tasks

WRT. Qualitative analysis of WRT performance did not show appreciable differences between the various protocol phases as a function of the two treatment cycles as regards accuracy, which was overall quite good (always up to 80%). The above described procedure, used to detect the best-fitting model of WRT accuracy, confirmed that the null model was the best among those tested.

As regards mean RTs, the model-fit analysis on the three parameters of the ex-Gaussian RT distribution revealed that the best-fitting model was, also in this case, the null model.

VWMT. The model-fit analysis of VWMT accuracy showed that the best-fitting model was that with the fixed factor Treatment Type (BIC = 4038.7 vs. null model BIC = 4040.5, ΔBIC = 1.8, a weak effect according to values as suggested by Raftery, 1995; $\beta = -0.37$, se 0.11, $p < .001$; Figure 2A), with the tDCS+CT condition generally showing a higher accuracy than sham+CT condition. The fact that the accuracy was quite similar in the post-treatment phase of both stimulation conditions reduces the possibility that the effect could be due to a time-dependent cognitive decline, and the qualitative inspection of VWMT accuracy (Figure 2A) highlighted a specific improvement of tDCS+CT condition in the treatment phase.

RT analyses revealed that the best-fitting model was that with the fixed factor Treatment Phase for the μ component, that is for the mean of the normal component of the ex-Gaussian RT distribution (BIC = 46652.95 vs. null model BIC = 46660.72, ΔBIC = 7.8, a strong effect, Figure 2C). In detail, μ analysis revealed a significant quadratic effect of treatment Phase ($\beta = 138.75$, *SE* 35.57, $p < .001$), with a pattern approximately similar for the two stimulation conditions, indicating an RT decrease during the treatment phase with respect to pre-treatment phase, followed by an increase in the following phase. Notably, the μ decrease in the treatment phase was primarily for the tDCS+CT condition; indeed the sham+CT condition did not show a noticeable change from pre-treatment to treatment phases.

PFT. The model-fit analysis showed that the null model was the best among those tested, proving that the mean number of produced words did not change appreciably as a function of the predictors.

CPT. Analysis of accuracy revealed that the null model was the best among those tested, therefore, performance correctness (always up to 80%, Figure 3A) did not significantly vary as a function of the predictors.

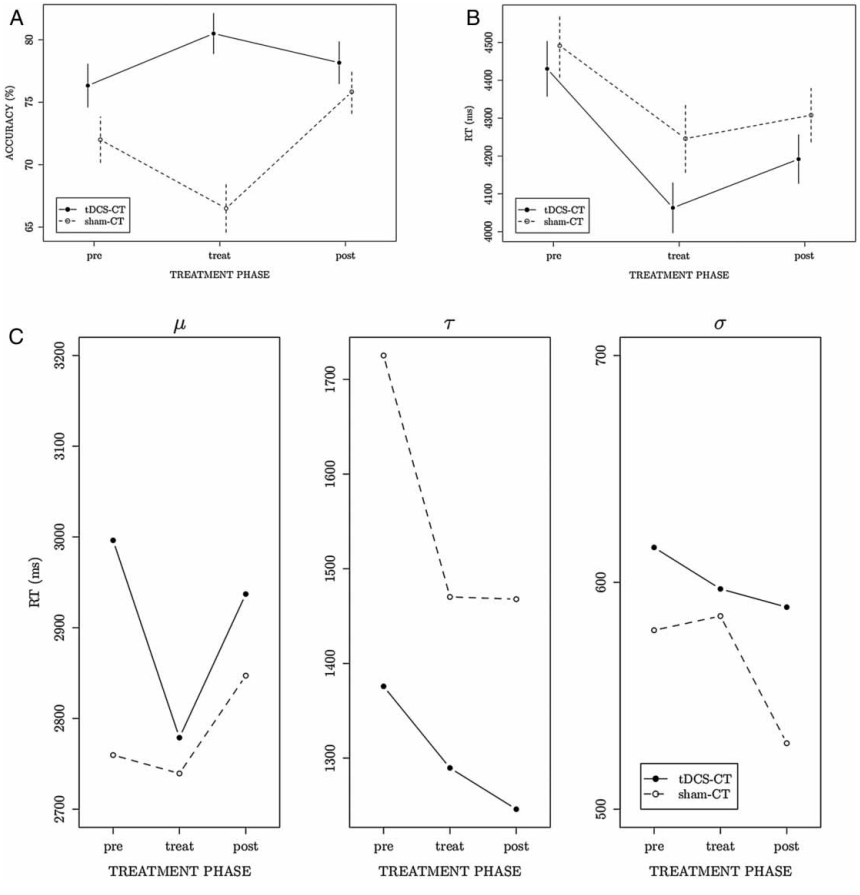

Figure 2. Results of Verbal Working Memory Task as a function of treatment type and treatment phase. (A) Mean percentages of accuracy, bars indicate *SE*. (B) Mean RTs, bars indicate *SE*. (C) Components (μ, τ, σ) of ex-Gaussian RT distribution.

The model-fit analysis on the three parameters of the ex-Gaussian RT distribution revealed that the best-fitting model was the model with the interactive effect of Treatment Phase and Treatment Type for τ parameter (BIC = 222425.3 vs. null model BIC = 222513.2, ΔBIC = 87.9, a very strong effect; significant linear effect for the interaction: $\beta = -0.36$, $SE = 0.03$, $p < .001$). Figure 3C highlights, for τ parameter, a decrease in the treatment phase compared to the pre-treatment phase for both treatment conditions, however, such decrease was maintained only in the sham+CT condition, whereas tDCS+CT condition showed higher τ values in the post- than in the pre-treatment phase. Interestingly, the qualitative comparison of μ and

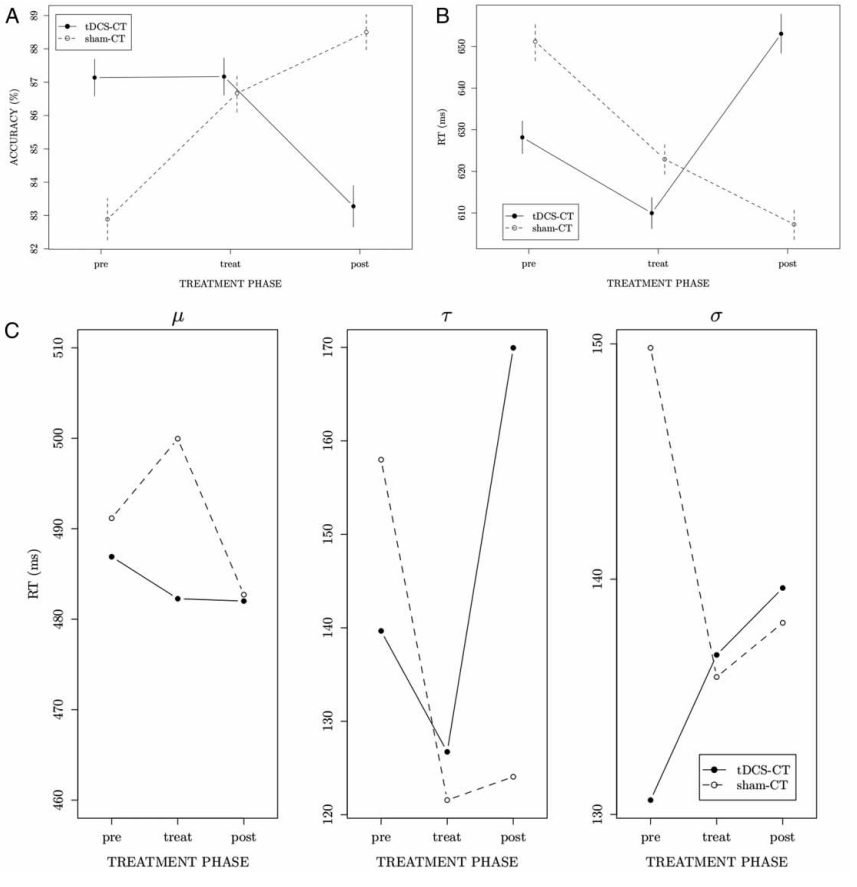

Figure 3. Results of Continuous Performance Task as a function of treatment type and treatment phase. (A) Mean percentages of accuracy, bars indicate *SE*. (B) Mean RTs, bars indicate *SE*. (C) Components (μ, τ, σ) of ex-Gaussian RT distribution.

τ revealed that, in the tDCS+CT condition, both μ and τ decreased from pre-treatment to treatment phase (with a following increase only for the τ component). Instead, in the sham+CT condition, μ and τ showed a nearly opposite pattern. Such qualitative differences, as well as the significant interactive effect of the predictors on τ, would have been neglected by analysing mean RTs only.

Neuropsychological tests

Table 1 shows the patient's scores for the *ENB-2* (Mondini et al., 2011) tests in the three neuropsychological assessments (t0, t1, t2) of each treatment

cycle, and the changes from one assessment to the previous one (i.e., $\Delta 1 = t1 - t0$ and $\Delta 2 = t2 - t1$). It is clearly evident that the tDCS+CT condition was more effective in improving, or not worsening, the performance compared with sham+CT condition. Indeed, the tDCS+CT condition was characterised by a significant improvement in three out of nine tests in the assessment immediately following the end of the stimulation sessions, and by a significant improvement in one test in the 1-month follow-up. The same stimulation condition was associated with only two significant performance reductions. In contrast, as regards the sham+CT cycle, it is noteworthy that there was a lack of any significant improvement in either t1 or t2, as well as a significant performance decline in many tests. These results suggest that the tDCS+CT condition could have slowed the cognitive decline of our patient over a period of approximately 3 months (from t1 of the first cycle, that is from the end of the first treatment phase, to t0 of the second cycle, that is to the baseline assessment of the second cycle).

DISCUSSION

The present case study provided mixed evidence for the therapeutic efficacy of tDCS combined with cognitive tasks as a training in AD. Indeed, on the one hand, the improvement for the trained tasks by the tDCS+CT condition was small, and not always in the expected direction. On the other hand, however, findings concerning the transfer effects on general cognitive functions showed that repeated tDCS sessions, combined with cognitive tasks, were more effective than the tasks alone in slowing down the cognitive decline of our AD patient over a period of approximately 3 months.

With regard to the trained CTs, in contrast with the only study reporting recognition memory improvements by stimulation of the LDLPFC (Boggio et al., 2009), we did not find any tDCS-induced effect on the task testing this function, possibly because of relevant differences between the two paradigms used (e.g., visual vs. verbal stimuli, online vs. offline stimulation, very short vs. long time-interval between encoding and recognition phases of the task). Such differences could, indeed, have made the two paradigms susceptible to be differently affected by the same kind of stimulation. Instead, by comparing our results with those achieved using an analogue paradigm (Ferrucci et al., 2008), we can infer a superior efficacy of the temporo-parietal stimulation used by Ferrucci and co-workers (2008), compared to the LDLPFC stimulation that we used, in improving recognition memory. In addition, there were no effects of treatments for the fluency task. In line with recent data (Penolazzi, Pastore, & Mondini, 2013) stressing the importance of electrode montages in determining stimulation-induced behavioural effects for a similar kind of task (i.e., semantic fluency), it is possible to

TABLE 1
ENB-2 test-scores in the various assessments of *tDCS+CT* condition and *sham+CT* condition

	tDCS+CT					*SHAM+CT*						
	t0	*t1*	*Δ1*	*t2*	*Δ2*	*t0*	*t1*	*Δ1*	*t2*	*Δ2*	*Mean* (SD)	*Cut- off*
Digit span	5	5	0	5	0	6	5	**−1**	5	0	6.11 (1.07)	5
Immediate memory	18	23	5	20	**−3**	21	21	0	19	**−2**	13.86 (4.05)	6
Delayed memory	19	22	3	24	2	23	25	2	26	1	18.21 (4.87)	9
Memory interf. (10 s)	3	6	*3*	5	−1	5	2	**−3**	3	1	7.39 (2.13)	5
Memory interf. (30 s)	1	3	2	1	−2	3	3	0	4	1	6.79 (2.20)	4
TMT-A	238	158	*−80*	165	7	103	124	**21**	145	**21**	43.37 (14.94)	66
TMT-B	342	391	49	406	15	267	495	**228**	534	39	104.22 (27.89)	149
Overlapping figures	19	15	**−4**	25	*10*	25	24	−1	21	−3	40.21 (9.96)	28
Clock Drawing	7	10	*3*	10	0	10	8	**−2**	9	1	9.16 (1.74)	7

ENB-2: Brief Neuropsychological Examination-2; TMT: Trial Making Test; t0 = baseline assessment; t1 = 1st post-treatment assessment; t2 = 2nd post-treatment assessment; Δ1: difference between t1 and t0 scores of the same cycle; Δ2: difference between t2 and t1 scores of the same cycle. For delta differences, the values in bold indicate a significant worsening of a given test compared with the previous assessment of the same cycle, the values in italic indicate a significant improvement of a given test compared with the previous assessment of the same cycle (worsening corresponds to negative values and improvement to positive values for all tests, except for TMTs, which are measured in sec and therefore need to be oppositely interpreted). * Mean (*SD*) and cut-off values (5^{th} percentile) of the sample matched for age and education to the patient.

hypothesise that the stimulation site chosen here is not the best one to reliably improve phonemic fluency. On the contrary, in accordance with the facilitative effects on healthy volunteers' working memory by anodal stimulation over the LDLPFC (Fregni et al., 2005), we found a significant accuracy improvement in the verbal working memory task for the tDCS+CT condition compared with the sham+CT condition, which appeared, however, limited to the treatment phase. Analyses of the ex-Gaussian RT distribution for the verbal working memory task revealed a decrease of μ parameter (i.e., the mean of the normal component of distribution) in the treatment phase with respect to the pre- and post-treatment phases. Although both treatment conditions seemed characterised by the μ decrement in the treatment phase, this effect was primarily for tDCS+CT condition, whereas the sham+CT condition did not show a noticeable change from pre-treatment to treatment phase. Therefore, both accuracy and RTs supported a facilitation (even if short-lived) of verbal working memory processes by tDCS+CT treatment. A somehow unexpected result was found in the continuous performance task, with a decrease of the τ parameter of ex-Gaussian RT distribution in the treatment compare to the pre-treatment phase for both treatment types, which was, however, maintained only in the sham+CT condition. This result is relevant in two respects. First, the improvements in the working memory task and in the general cognitive functions (assessed by neuropsychological tests) were likely not mediated by an enhancement of sustained attention. Second, although the result did not prove a strong interfering effect of tDCS+CT condition on the task (the τ increase in this condition was only related to the post-treatment phase), it highlights a relevant issue recently raised in a study on healthy volunteers (Iuculano & Kadosch, 2013): that is, the possibility that a given cognitive enhancement through non-invasive brain stimulation can occur at the expense of other cognitive functions. Although, as mentioned, this was not strictly the case in our study, this risk needs to be fully considered, especially when using non-invasive brain stimulations with patients showing deficits in many cognitive domains, like AD patients. A possible way to control for this risk is to assess as many cognitive processes as possible, in single-session protocols, in order to have a clear picture of which processes can be improved, which are not affected, and which (if any) can be impaired by a given stimulation protocol. Then, only evaluating stimulation-induced costs and benefits, it could be decided whether it is worth starting a repeated session treatment using the same parameters, or looking for other better stimulation parameters.

On the whole, therefore, the present tDCS+CT treatment showed a limited effect on the trained tasks. Apart from the already discussed role of the stimulation parameters (i.e., electrode montage, number of sessions, etc.) in determining the behavioural results of a given cognitive task, this limited effect on CTs could have been caused by many different factors. Among these, one

possibility is that, repeatedly performing the CTs for 10 sessions, in the pre-treatment phase of the first cycle, had almost exhausted the patient's reserve of recovery, so that it became very difficult for further considerable improvement in the following treatment phase in which the tDCS was added to the CTs. Although this would mean that the tDCS was not able to determine an additional improvement to that induced by repeatedly practising the tasks (except for the verbal working memory task), it does not necessarily imply that combined treatments similar to ours are definitely ineffective. Indeed it might be that changes in the features of both tDCS and cognitive training can boost more extensive effects.

Although analysis of the trained tasks showed that tDCS combined with cognitive training was able to slightly improve verbal working memory only, a very relevant aspect of our findings is that two out of four tasks showed treatment-induced effects for specific parameters of ex-Gaussian RT distribution, which would have been missed by simply focusing on mean RTs (see the comparison between Figures 2B, 2C and 3B, 3C). Such parameters can sometime be differently affected as a function of the treatment type. Since these parameters have often, although not unequivocally, been interpreted as reflecting different kinds of processes (i.e., more automatic processes for the μ component vs. more controlled processes for τ component, see Matzke & Wagenmakers, 2009), their analysis can be extremely helpful in characterising which processes could be specifically affected by the treatment. Therefore, from a methodological point of view, the present findings strongly recommend considering the ex-Gaussian RT distribution, because the analysis of its parameters allows detection of very specific effects of stimulation, that could be obscured by performing only standard statistics on mean RTs.

Focusing on the most important outcome of the present study, that is the performance in neuropsychological tests evaluating possible transfer effects, we found a clear-cut superior efficacy of the tDCS+CT condition in improving, or at least not worsening, the performance, compared with the sham+CT condition (characterised, instead, by a general worsening in most tests). Although it could be argued that applying real stimulation first might be less than ideal, because the worsening in many tests during the sham+CT cycle could merely represent a cognitive decline somewhat expected in degenerative dementia, this possibility can be reasonably excluded, since the comparison between t2-scores of the tDCS+CT condition and the t0-scores of sham+CT condition (Table 1), collected about 2 months later, did not show any significant reduction. Therefore, there is no reason to suspect that the progression of the degenerative disease in a 1-month interval (from t0 to t1, or t1 to t2 of both cycles) would be faster than the progression in a 2-month interval (from t2 of tDCS+CT to t0 of sham+CT). The possibility that the effects of the first treatment cycle could survive beyond the first

intervention (thus influencing the effects of the second cycle) is also unlikely, as these potential outlasting tDCS effects should have worked against the possibility of observing a decreased performance in the sham cycle (which was instead what was found). Finally, even the possibility that the improvements in the tDCS+CT condition could be due to practice effects (i.e., repeated assessments with the same test material, patient getting used to the protocol and the experimental environment) is highly unlikely, because in the last assessments (i.e., t1 or t2 of sham+CT), which should benefit to a greater extent from practice effects, performance worsened in many tests. The most plausible interpretation of the neuropsychological data is that tDCS+CT treatment had a superior efficacy over sham+CT treatment in slowing cognitive decline, with effects lasting approximately 3 months. Although the few actual improvements were mostly limited to the first post-treatment assessment (i.e., t1 of tDCS+CT), the lack of significant performance reductions up to the first assessment of sham+CT condition (i.e., t0 of sham+CT) pointed to a slowing down of cognitive decline specifically induced by the synergetic effects of tDCS and CTs. The present data do not allow us to establish whether the general cognitive improvement in the tDCS+CT cycle was due to the stimulation only or to possible combined effects of tDCS and CTs, but the latter, when administered alone, did not seem able equally to preserve the patient from a relatively quick cognitive decline. With respect to the mechanisms involved in the transfer effects on general cognitive functions, although we cannot exclude the intervention of pivotal cognitive functions not specifically assessed (e.g., planning, selective attention, etc.), it is possible to hypothesise a specific crucial involvement of working memory in mediating the generalisation effects, given the improvements induced by the tDCS+CT condition in the working memory task.

Despite the limitation imposed by the single case study, the results of our patient's general cognitive functions are encouraging, showing that the synergetic use of tDCS and cognitive tasks for 10 daily sessions can induce a slowing down of cognitive decline, which could not be obtained by performing the same cognitive tasks alone. However, it should be taken into account that the promising results we found in the neuropsychological tests are not in line with recent data collected in a group of AD patients, showing no additional improvement, with respect to the placebo condition, by repeated anodal stimulations of the LDLPFC (i.e., the same area that we stimulated), associated with an on-line memory training (Cotelli et al., 2014). It is worth reiterating that these inconsistencies may arise from differences in both the paradigms used (i.e., online vs. offline stimulation, very different kinds of cognitive training and tasks used to assess stimulation effects, etc.), and the individual characteristics of the patients undergoing stimulation (i.e., age, dementia severity, etc.). Nevertheless, based on data showing that stimulation parameters similar to ours cannot be considered reliably effective

in ameliorating some cognitive deficit in AD, we acknowledge that the present preliminary results require further support to be generalised. To conclude, current literature indicates limited and sometimes puzzling effects of tDCS in AD, yet some positive evidence has been provided (by our single case, but see also Boggio et al., 2009, 2012; Ferrucci et al., 2008). Therefore we believe that research on this technique should persist in the search for the optimal stimulation parameters and clinical paradigms that enable tDCS to boost improvements in AD.

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