Transcranial direct current stimulation over multiple days improves learning and maintenance of a novel vocabulary

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Short title: Impact of tDCS on language learning

Abstract

Introduction: Recently, growing interest emerged in the enhancement of human potential by means of non-invasive brain stimulation. In particular, anodal transcranial direct current stimulation (atDCS) has been shown to exert beneficial effects on motor and higher cognitive functions. However, the majority of tDCS studies have assessed effects of single stimulation sessions that are mediated by transient neural modulation. Studies assessing the impact of multiple stimulation sessions on learning that may induce long-lasting behavioural and neural changes are scarce and have not yet been accomplished in the language domain in healthy individuals.

Method: The present study probed the potential of atDCS to enhance language learning over multiple days by employing an explicit word learning paradigm. Forty healthy young participants were randomized to learning with either simultaneous atDCS or sham stimulation (N=20/group; comparable regarding demographic variables and neurocognitive status). All participants acquired a novel vocabulary (familiar and novel object picture - non-word pairs) over five consecutive days. Two memory tasks (free recall; forced choice recognition tasks) were administered immediately after each training session. A one-week follow-up tested the maintenance of learning success.

Results: Linear mixed effects model analysis revealed superior learning during atDCS compared to sham stimulation for both familiar and novel objects. atDCS yielded a steeper learning curve and significantly more pronounced learning at the end of the training during the recall task. During the recognition task, the atDCS-group reached ceiling levels earlier and overall learning success was greater. For both tasks, beneficial atDCS-effects were maintained during the follow-up assessment.

Conclusions: The present study provides direct evidence that atDCS administered during multiple learning sessions facilitates language learning and that effects are maintained over time. This study contributes important novel information about the extent of stimulation effects in the healthy brain, thereby highlighting the potential of atDCS to enhance language recovery after stroke.

Key words: brain stimulation; transcranial direct current stimulation; language functions; learning; longitudinal design

1. Introduction

Neuroplasticity refers to dynamic structural and functional central nervous system reorganization due to internal or external demands and is recognized as the main physiological basis for adaptive behavioural changes (Pascual-Leone et al., 2005). Recently, growing interest emerged in the enhancement of neuroplasticity by means of non-invasive brain stimulation techniques like transcranial direct current stimulation (tDCS; Flöel and Cohen (2010)) to improve human performance and learning. During tDCS, weak electrical currents are applied to the scalp to modulate excitability of underlying neural populations (Nitsche et al., 2003). The most consistent beneficial effects on motor and cognitive functions have been reported for anodal stimulation (atDCS; Kuo and Nitsche (2012)) that facilitates firing of task-specific neuronal populations. Moreover, due to its excellent safety profile and effective placebo ("sham") stimulation option (Gandiga et al., 2006), atDCS has become increasingly popular in research and clinical settings (Flöel, 2012; Kuo and Nitsche, 2012).

In the language domain, a number of studies have demonstrated that atDCS administered to left perisylvian cortices improves word-retrieval and lexical processing when administered prior to or during task performance (Cattaneo et al., 2011; Flöel, 2012; Meinzer et al., 2012; Meinzer et al., 2013; Peretz and Lavidor, 2013; Sparing et al., 2008). In addition, atDCS has also improved vocabulary (Fiori et al., 2011; Flöel et al., 2008) and artificial grammar learning (de Vries et al., 2010). In the latter studies, training was accomplished across several blocks during a single stimulation session and superior learning immediately after the training was reported for anodal compared to sham or inhibitory cathodal tDCS. Those short-term effects can be explained by transient modulation of resting-membrane potentials that outlast the stimulation only for short periods of time (Nitsche et al., 2003). As a result, immediate beneficial effects on language functions may not be maintained over time (Flöel et al., 2008).

However, with regard to potential clinical applications, stimulation protocols that induce long-lasting behavioural modifications are of utmost importance. Indeed, long-lasting modifications are feasible with tDCS, since repeated stimulation sessions result in modification of post-synaptic connections similar to long-term potentiation which is critical for learning and neuroplasticity (Stagg and Nitsche, 2011). However, such studies have not yet been accomplished in the language domain in healthy individuals and so far, only few studies assessed the impact of repeated atDCS sessions on learning in other domains. For example, two recent studies demonstrated faster and better motor skill learning after a one-week training period with atDCS compared to training alone (Reis et al., 2009; Zimerman et al., 2013). Superior training outcome was maintained for up to three months (Reis et al., 2009). For higher cognitive functions, Cohen Kadosh et al. (2010) demonstrated enhanced numerical learning after six days of training with atDCS compared to sham or inhibitory cathodal tDCS and gains were maintained for six months. Another recent study, however,

failed to demonstrate maintenance of immediate atDCS effects on cognitive functions after 10 days of computer assisted training (Martin et al., 2013). This study highlights the pressing need to further explore the extent of potential beneficial effects of repeated atDCS sessions in healthy subjects.

Based on the above studies in healthy individuals and also previous studies in stroke patients showing that the combination of atDCS and motor therapy resulted in enhanced outcome compared to treatment alone (for review see Reis and Fritsch, 2011), it has been suggested that atDCS may be valuable as an adjunct treatment for post-stroke language disorders (aphasia, Holland and Crinion, 2012). Indeed, overall beneficial effects of atDCS (compared to sham or cathodal tDCS) have been demonstrated in preliminary pilot trials (Baker et al., 2010; Fiori et al., 2011; Flöel et al., 2011). However, these previous studies also yielded highly variable stimulation effects in individual patients, possibly due to the unknown relationship between language system reorganization after stroke and the stimulation site (for further discussion see Flöel, 2012, 2013; Meinzer et al., 2011). This emphasizes the urgent need for proof-of-principle studies in healthy participants, to explore the extent of tDCS-effects on language learning under highly controlled experimental conditions. Indeed, it has been suggested that studies of new word learning in healthy participants can be informative regarding how to optimize language re-learning in stroke patients (Basso et al., 2001). Therefore, the present study employed an explicit new word learning paradigm (Whiting et al., 2007, 2008) scheduled over five consecutive days either with concurrent atDCS or sham stimulation. By examining learning of new names associated with both familiar and unfamiliar objects (Laine and Salmelin, 2010), we also examined the linguistic specificity of atDCS effects on learning. In addition, long-term effects of the stimulation were assessed during a one week follow-up period. Based on previous studies in other domains (Cohen Kadosh et al., 2010; Reis et al., 2009), we hypothesized that training plus atDCS would result in more pronounced language learning and maintenance of stimulation effects than training with sham stimulation.

2. Methods

2.1. Study overview: The study employed a between-subjects randomized sham-controlled single-blind design and was conducted at the Charité University Hospital in Berlin, Germany. Participants underwent five learning sessions during which they acquired a novel vocabulary using an established explicit associative learning procedure (Whiting et al., 2007, see below for details; 2008). We deliberately chose an explicit learning paradigm because it more closely resembles current treatments for word-retrieval impairments in aphasia (Nickels, 2002) than previous implicit new word learning paradigms (Flöel et al., 2008).

Figure 1 illustrates the design of the present study. Training sessions were scheduled over five consecutive

days and half of the participants received either atDCS or sham stimulation during learning. Each daily learning session comprised an acquisition period, during which participants were simultaneously presented with 120 pairs of object pictures and non-words on a computer screen. Prior to the acquisition phase, participants were instructed to memorize each object – non-word pair and informed that they would be required to complete two memory tasks after the end of each daily training session. The memory tasks comprised a "recall task" during which participants were presented with the previously shown 120 pictures and asked to type the correct non-word name of each object using a computer keyboard. A subsequent "recognition task" required a forced choice decision between two non-words that were displayed simultaneously with a given object. A follow-up session (only recall and recognition tasks) was completed one week after the last day of the training and tested the maintenance of the novel vocabulary.

2.2. Participants: Forty healthy young volunteers (44 that were initially screened, four did not fulfil inclusion criteria, see below) participated in this study (24 women, 16 men, age range 18-32 years, mean±SD 23.9±3.6). Participants were all right-handed (Edinburgh Handedness Inventory score ≥50; Oldfield (1971)) native German speakers with normal or corrected-to-normal vision. Participants with a history of chronic or acute neurologic, psychiatric, or medical disease, a family history of epilepsy, current pregnancy, cardiac pacemaker or previous surgery involving implants to the head were excluded from the study. Participants did not take any acute or chronic medication (other than contraceptives) or recreational drugs. Prior to study inclusion, written informed consent was acquired and participants were reimbursed with €120 after study completion. The study was approved by the local ethics committee and conducted in accordance with the Helsinki declaration. Participants were randomized by sex to the two stimulation groups (see **Table 1** for additional information; groups were comparable with regard to demographic characteristics and cognitive status and number of foreign languages fluently spoken).

2.3. Cognitive screening: A comprehensive neuropsychological test battery was administered prior to study inclusion. This battery tested verbal short- and long-term memory (digit span, Härting et al. (2000); Verbal Learning and Memory Test, VLMT, Helmstädter et al. (2001)), visual-spatial abilities and memory (Rey-Osterrieth Complex Figure Test, Shin et al., 2006), executive functions (Regensburger Verbal Fluency Test, RWT, Aschenbrenner et al. (2001); Trail Making Test, TMT, (2004)), verbal IQ and vocabulary (Synonym-Antonym Selection and Classification Test, SASKA, Riegel (1967); Vocabulary Test, MWT, Lehrl (2005)). The test battery was used to assure normal cognitive functions and to assess whether stimulation groups would differ with regard to cognitive status (see **Table 1** for details). All participants performed within age-appropriate norms.

2.4. Transcranial direct current stimulation: tDCS was administered through a battery-driven direct current stimulator (DC-Stimulator Plus, NeuroConn GmbH, Ilmenau, Germany). Each daily session was carried out between 9 a.m. – 4 p.m. according to the preferences of each participant. For individual participants, the

same time of day was scheduled for each daily session. The electrodes were inserted into synthetic sponges soaked in saline solution and attached to the scalp using rubber bands. The anode (5×7 cm^2) was centred over the left posterior temporo-parietal junction. This area has been shown to be important for successful new word learning in healthy individuals and patients with post-stroke language impairment (e.g., Cornelissen et al., 2003; Davis and Gaskell, 2009; Hulten et al., 2010; Laine and Salmelin, 2010). Moreover, atDCS administered to this area resulted in improved implicit new word learning in a previous study of our workgroup (Flöel et al., 2008). Electrode position was determined using the international 10-20 EEG system (position Cp5). The cathode (10×10 cm^2) was placed over the contralateral supraorbital region. The large size (10x10 cm2) of the reference electrode renders the stimulation over the contralateral orbitofrontal cortex ineffective without compromising the stimulation effect underneath the active electrode (Nitsche and Paulus, 2011).

A constant direct current of 1 mA was applied during atDCS. Stimulation was turned on approximately two minutes prior to the acquisition phase and continued until the end. In the anodal group, stimulation was administered for 20 minutes which assured that active stimulation was administered during the entire learning session. As in previous studies (Flöel et al., 2008; Meinzer et al., 2012) the current was increased and decreased in a ramp-like fashion over 10 sec at the beginning and at the end of the stimulation. During sham, the procedure was exactly the same, but the current was ramped down after 30 sec. The latter procedure does not modulate neural functions but elicits a tingling sensation on the scalp to ensure efficient blinding of the participants with regard to the stimulation conditions (Gandiga et al., 2006). After completion of each daily learning session, tDCS electrodes were removed from the participants' head before participants continued with the recall and recognition tasks.

2.5. Stimulus selection and procedure

2.5.1. Object pictures: We chose stimuli that had previously been used to assess the impact of (pharmacological) intervention on explicit new word learning (Whiting et al., 2007, 2008). Specifically, 120 black and white pictures of objects were selected, including 60 standardized pictures of real objects ("familiar objects", Snodgrass and Vanderwart (1980)) and 60 "novel objects" from the ancient farming equipment paradigm, i.e., pictures of artifacts used in Finnish households in medieval times unknown to modern-day humans (Cornelissen et al., 2003; Laine and Salmelin, 2010). The latter stimuli were chosen based on a pilot study, during which twenty participants (mean±SD 26.9±3.3 years; 10 women) not involved in this study rated familiarity of 183 novel objects (i.e., they were asked to provide up to three associations elicited by each object, if any). Stimuli were excluded from further consideration if they elicited the same association in at least 3/20 participants, and we selected the 60 objects with the lowest familiarity score. The main reason to include the two different object types was to enhance variability in the training sets and

to increase task difficulty, thereby preventing ceiling effects. In addition, we were also interested in the linguistic components of learning that are modulated by tDCS. Specifically, previous studies examined implicit (Flöel et al., 2008) or explicit (Fiori et al., 2011) learning of new lexical labels for familiar objects. Therefore, in both studies orthographic representations were associated with existing semantic and conceptual representations. In contrast, this study also allowed us to determine the effects of atDCS on learning names of unfamiliar objects where no existing semantic representation is available.

2.5.2. Non-words: 180 pronounceable non-words composed of 4-6 letters were used in the present study (120 were used during learning and the memory tasks, the remaining 60 as untrained distractors during the recognition task, please see below). All were in accordance with phonotactic rules of German but had no associated meaning and were written with an initial capital letter (i.e., in line with German orthographic rules for nouns). The non-words were selected based on the above pilot study during which participants were also asked to list associations for 322 non-words with known German words and ratings of emotional valence (scale of 1-7; very unpleasant=1, very pleasant=7). The final set of 180 non-words comprised only words with an average of <1 associations and neutral valence (mean±SD associations: 0.24±0.46; valence 3.97±0.54). The final set was divided into three sets of 60 words each, that were matched for word length (mean±SD letters Set1 4.85±0.79, Set2 4.85±0.77, Set3 4.81±0.81, $F(2,177)=0.04$, $p=0.96$), valence (Set1 4.03±0.57, Set2 3.96±0.58, Set3 3.94±0.53, $F(2,177)=0.61$, $p=0.54$), number of associations (Set1 0.21±0.45, Set2 0.28±0.49, Set3 0.22±0.41, $F(2,177)=0.66$, $p=0.51$) and distribution of initial letters. To ensure a variation of object - non-word pairs, six different lists were generated (i.e., non-word Set1 + familiar objects, Set2 + novel objects, Set3 as distractor during the recognition task, etc., in all possible combinations). Sets1-6 were randomly assigned to the participants. In addition, non-words were randomly assigned to the respective objects in each participant to assure maximal variation of pairings.

2.6. Learning and memory tasks: Presentation software (Neurobehavioral Systems, Version 14.8.) was used for stimulus presentation and recording of responses. During the acquisition phase, pictures and words appeared simultaneously on a black screen for four seconds. Pairs were presented in five blocks (24 trials/block) with a short gap in between trials (500 msec). After each block a short break was inserted and participants could proceed to the next block at their own pace. The same number of familiar and novel objects were included in each block (N=12). Duration of the acquisition phase was approximately 12-14 minutes, depending on the length of the individually chosen breaks between blocks. Afterwards, electrodes were removed and participants immediately continued with the two memory tasks. During both tasks, the entire set of trained items were tested (N=120).

Participants first completed the recall task (duration ~15 min) and were encouraged to always provide an answer, even if based on guessing. There was no time limit, and the participants could correct typing errors.

Each trial was completed by pressing the return key. Subsequently, participants performed the recognition task. Here, each picture was simultaneously presented with two non-words for 4 seconds (see Figure 1). The participants were required to choose the correct non-word within this time frame by pressing the left or the right mouse button (total duration ~5-6 minutes). In 50% of the trials the correct target non-word was presented with either an incorrect non-word that had been included in the training (i.e., a word that had been paired with a different object) or a new word that had not been used during training (i.e., 60 non-words from the third list). Positions (right/left side on screen) of correct and incorrect non-words were counterbalanced. As in the learning sessions, the two memory tasks comprised 5 blocks of 24 trials and a short break in between blocks. The dependent variable was the percentage of correct responses during each daily testing session. For the recall task, only 100% correct responses were scored. Participants did not receive feedback about their performance until after the end of the study.

2.7. Mood ratings: Before and after each learning session, participants rated their emotional state using the Positive and Negative Affect Schedule (PANAS, Watson et al., 1988). The PANAS assesses positive and negative affect (10 items each) on a scale ranging from 1-5 with higher values describing more positive or negative feelings. In addition, participants evaluated the intensity of potential adverse effects due to the stimulation using a brief 10-item questionnaire at the end of the study (see Table 2). This questionnaire included symptoms like burning, itching, and headache. Severity of symptoms was rated on a scale from 1-5 (1=none, 2=mild, 3=moderate, 4=intense, 5=very intense).

2.8. Statistical analysis: We calculated linear mixed models (Verbeke and Molenberghs, 2000), separately for the two outcome measures (i.e., percentage correct responses during recall and recognition tasks), where the five time points during the learning phase (factor TIME) were level-one units nested in the different individuals, who were level-two units. Random intercept models tested differences between the two stimulation conditions (atDCS, sham) which included OBJECT TYPE as covariate (familiar objects; novel objects). Additionally, we included a squared centred time variable ($TIME^2$) to test if there was a curvilinear learning-curve. The interaction TIME x STIMULATION assessed whether the slopes of the learning-curves differed between the stimulation groups. An interaction term of $TIME^2$ x OBJECT TYPE was included to test whether the shape of the learning-curves differed for the two object types. Finally we tested whether the stimulation group effect was different for familiar and novel objects by including an interaction term OBJECT TYPE x STIMULATION. (model parameters for recall/recognition tasks $day_{1\text{-}5}$: N=40 participants each, 400/392 test values, random effects, variance between subjects: β= 0.019/0.003, standard error (SE)= 0.005/0.001, Z= 4.03/3.86; residual variance: β= 0.015/0.003, SE= 0.001/0.0003, Z= 13.3/13.2, all p< 0.0001). Differences between the stimulation groups on day_5 (i.e., the last day of training) and the follow-up assessment one week later (day_6) were also tested, separately for recall and recognition tasks. This analysis assessed the long-term maintenance of the learning success and whether a potential decrease in

vocabulary knowledge after one week was steeper in the anodal stimulation group than in the sham group (model parameters for recall/recognition tasks $days_{5-6}$: N=40 participants each, 160/160 test values, random effects, variance between subjects: β= 0.043/0.001, standard error (SE)= 0.010/0.0005, Z= 4.08/3.71; residual variance: β= 0.012/0.001, SE= 0.002/0.0002, Z= 7.62/7.62, all p< 0.0001). Additional models tested for differential effects of the stimulation on positive and negative affect ratings.

3. Results

Participants in both stimulation groups tolerated the stimulation well. No serious adverse events were reported and only two symptoms were rated for mild-moderate effects in both groups (tickling and itching; see **Table 2**). No significant differences were evident between stimulation groups with the exception of "itching" which was rated as slightly more pronounced in the group that received atDCS. In line with previous studies that used 1 mA (Gandiga et al., 2006), participants could not differentiate between atDCS and sham stimulation as indicated by responses on a post-study questionnaire.

3.1. Impact of atDCS on learning

Figure 2 illustrates acquisition of the novel vocabulary across the five training sessions and the maintenance of the training effects as assessed during the follow-up session one week after the end of the training for both stimulation groups. Effects are displayed separately for familiar and novel objects and recall and recognition tasks. Please **Table 3** for details of raw data and percentages.

3.1.1. Recall of familiar and novel objects (learning phase, day_{1-5}): Participants successfully acquired the novel vocabulary across the five learning sessions (significant positive effect of TIME β= .14, SE= .006, t(354)= 22.28, p< .0001). For the recall task the learning curve was curvilinear (positive coefficient of $TIME^2$ β=.01, SE= .005, t(354)= 2.65, p= .008) and the significant interaction of $TIME^2$ x OBJECT TYPE indicates that the type of curve is different for familiar and novel objects (β= -.03, SE= .007, t(354)= -4.58, p< .0001). For familiar objects the curve was hill-shaped due to the ceiling-effect by the end of the training, whereas there was a more linear increase for novel objects.

The effect of the STIMULATION in itself was not significant (β= .01, SE= .05, t(354)= .21, p= .84) but the interaction TIME x STIMULATION was significant and positive (β= .04, SE= .009, t(354) = 4.35, p< .0001) indicating that the anodal group exhibited a steeper increase in correct answers over time than the sham group. Post-hoc t-tests showed that by the end of the training, overall learning success during the recall task was more pronounced in the group that received atDCS (familiar objects day_5: t(38)= 2.32, p= .02; novel objects: day_5: t(38)= 2.25, p= .03). Learning of familiar objects was more pronounced than learning of novel objects as indicated by a significant effect of OBJECT TYPE (β= 0.27, SE= .02, t(354)= 11.84, p< .0001).

However, the interaction STIMULATION x OBJECT TYPE was not significant (β= .01, SE= .02, t(354)= .44, p= .66), which means that more pronounced learning during atDCS was evident for familiar and novel objects alike.

3.1.2. Recall of familiar and novel objects (maintenance of training gains, day_{5-6}): Compared to the last day of training both groups demonstrated a small (~10%) but significant drop in performance at one-week follow-up assessment (day_6, **see Figure 2**; TIME β= -.11, SE= .02, t(116)= -4.54, p< .0001). However, this effect was comparable in the two stimulation groups (non-significant interaction of TIME x STIMULATION (β= -.02, SE= .03, t(116)= -.64, p= .52). Overall, more pronounced learning success immediately after training (day_5) during atDCS was maintained at the follow-up assessment (STIMULATION β= .17, SE= .07, t(116)= 2.41, p= .017) and the drop of performance was comparable for familiar and novel objects (STIMULATION x OBJECT TYPE β= -.02, SE= .03, t(116)= -.63, p= .53). Again, learning of familiar objects was more pronounced than learning of novel objects as indicated by a significant effect of OBJECT TYPE (β= .27, SE= .02, t(116)=11.12, p< .0001).

3.1.3. Recognition of familiar and novel objects (learning phase, day_{1-5}): Similar to the recall task, recognition of both object types improved significantly across the five training sessions (TIME β= .08, SE= .003, t(346)= 25.59, p< .0001) and learning followed a hill-shaped learning curve ($TIME^2$ β= -.02, SE= .002, t(346)= -7.51, p< .0001). Overall, more pronounced learning occurred for familiar compared to novel objects (OBJECT TYPE β= .14, SE= .01, t(346)= 13.05, p< .0001) and the significant interaction of $TIME^2$ x OBJECT TYPE indicates that the learning curve was steeper for familiar objects (β= -.01, SE= .004, t(346)= -3.18, p= .0016).

The slopes of the learning curves were comparable in the two stimulation groups (interaction TIME x STIMULATION β= .00004, SE= .004, t(346)= .01, p= .993). However, participants who received atDCS showed more pronounced overall learning success (STIMULATION β= .06, SE= .02, t(346)= 3.06, p= .002) and this effect was more pronounced for novel objects than for familiar objects (interaction STIMULATION x OBJECT TYPE (β= -.03, SE= .01, t(346)= -2.69, p= .008). Learning success at the end of the training (day_5) was superior in the group that received atDCS for both object types (familiar objects day_5: t(38)=2.29, p= .03; novel objects: day_5: t(38)=2.81, p= .007).

3.1.4. Recognition of familiar and novel objects (maintenance of training gains, day_{5-6}): As for the recall task, a significant drop of performance from the last day of training was evident during the follow-up assessment one week after the training (TIME β= -.02, SE= .008, t(116)= -3.08, p= .003), however, this drop was comparable in both stimulation groups (TIME x STIMULATION β= .01, SE= .01, t(116)=1.06, p= .291). Significant differences between the stimulation groups at the end of the training (day_5) were maintained one week after the end of the training for both object types (STIMULATION β= .06, SE= .02, t(116)= 3.49, p=

.0007) and this effect was more pronounced for novel objects than for familiar objects (interaction STIMULATION x OBJECT TYPE β= -.034, SE= .01, t(116)= -3.84, p= .0002). Overall, more pronounced learning occurred for familiar compared to novel objects (OBJECT TYPE β= .07, SE= .008, t(116)= 8.52, p< .0001).

3.2. Mood ratings: Mean negative and positive mood rating ratings obtained prior to and after each daily training session are displayed in **Table 4**. Mixed effects models showed that there was no change in mood ratings over time in either group (TIME negative/positive scale β= -.015/-.007, SE= .01/.02, t(354)= -1.49/-.36, p= .137/.717), no significant effect of STIMULATION (negative/positive scale β= .06/-.20, SE= .07/.18, t(354)= .79/-1.13, p= .427/.258) and mood ratings did not change significantly from pre- to post assessments (negative/positive scale β= -.001/.08, SE= .03/.06, t(354)= -.04/1.33, p= .972/.185). In addition, anodal stimulation did not affect changes in mood over time as indicated by the non-significant interaction of TIME x STIMULATION (negative/positive scale β= -.02/-.01, SE= .01/.03, t(354)= -1.42/-.50, p= .157/.618) and pre-post ratings were comparable in both stimulation groups (negative/positive scale x STIMULATION β= -.04/.15, SE= .04/.08, t(354)= -1.09/1.91, p= .277/.057). Random effects for these models were: variance between individuals: β= .03/.26, SE= .009/.06, Z=3.9/4.1, both p< .0001; residual variance: β= .04/.16, SE= .003/.01, Z=13.3/13.3, both p< .0001.

4. Discussion

The present study employed an explicit novel word learning paradigm as a model to assess potential beneficial effects of multiple atDCS sessions on language learning. Overall, our results show that learning was faster and more pronounced during atDCS compared to sham stimulation for both tasks and object types. Moreover, superior gains were maintained for at least one week after the end of the training. The two stimulation groups were comparable with regard to demographic and neuropsychological characteristics and mood ratings were not affected by the stimulation, therefore, our findings can be attributed to the stimulation conditions. The positive results of this study emphasize the potential of atDCS to enhance treatment outcome in clinical conditions like post-stroke aphasia.

4.1. Impact of atDCS on language learning: In line with studies in other cognitive domains (Turi et al., 2012), the majority of studies that have assessed the impact of atDCS on language processing and learning in healthy adults report beneficial effects of the stimulation on behavioural performance (for review see Flöel, 2012). Moreover, three recent studies that employed functional magnetic resonance imaging and simultaneous stimulation of anterior language areas during word-retrieval tasks suggested that atDCS results in more efficient neural processing in language-related functional brain networks (Holland and Crinion, 2012; Meinzer et al., 2012; Meinzer et al., 2013). However, only one study so far addressed whether more pronounced learning induced by a single session of atDCS is maintained over time (Flöel et al., 2008). Given that immediate effects of the stimulation are mediated by transient modulations of

resting-membrane potentials (Stagg and Nitsche, 2011), it is not surprising that no lasting stimulation effects were observed in this study.

On the other hand, long-term maintenance of superior learning during atDCS was demonstrated for motor skills (Reis et al., 2009) and numerical abilities (Cohen Kadosh et al., 2010) when repeated learning sessions were combined with stimulation. The results of the present study extend these positive findings for the first time to the language domain. First, both stimulation groups acquired the novel vocabulary well, e.g., by the third day of training performance during the easier recognition task was >75% correct answers in both groups. This emphasizes that participants were attentive and motivated to perform the task. Second, in participants that received additional atDCS during learning, gains were more pronounced and the learning curves were steeper for both object types during the recall task. Even during the less demanding forced choice task, the atDCS group performed better for novel objects and reached ceiling levels earlier for familiar objects. Third, superior gains attained by the end of the training were maintained at least one week after the end of the training. Importantly, we also demonstrated that atDCS did not affect the rate of forgetting during the follow-up assessment compared to sham stimulation. Thus, as in previous studies in the motor domain (Reis et al., 2009), faster and more pronounced learning under atDCS does not come at the cost of more pronounced performance declines. Moreover, given that the two memory tasks were administered immediately after the end of the stimulation (i.e., during a phase where atDCS-effects are thought to persist), the maintenance of atDCS-induced performance gains one week after the end of the training demonstrates that the stimulation did not only result in immediate performance enhancements. These results are also in line with a previous study in aphasia rehabilitation, where individual gains during atDCS were maintained beyond the end of the training period (Flöel et al., 2011).

Similar to previous studies that employed the same stimuli to study effects of pharmacological intervention on training success (Whiting et al., 2007, 2008) beneficial stimulation effects were found for familiar and unfamiliar objects alike. Therefore, despite differences between the object types and learning required (i.e., linking a new word form with a familiar object which already has a lexical associate versus acquisition of a new object and associating it with a novel word, where one cannot rely on an existing lexical-semantic representation (Davis and Gaskell, 2009; Laine and Salmelin, 2010), the results of the present study emphasize facilitation of a common process, inherent to learning associations between novel word-forms and both object types.

4.2. Stimulation location: In the present study we chose the posterior temporo-parietal junction (Cp5 of the 10-20 EEG system) as target region for stimulation. This area was selected as its stimulation yielded superior learning in two previous studies that assessed immediate effects of atDCS on learning using similar training paradigms (Fiori et al., 2011; Flöel et al., 2008). Our stimulation site overlaps with “Wernicke's

area" which has been implicated with word production and phonological retrieval processes (Davis and Gaskell, 2009; Indefrey and Levelt, 2004). However, due to the relatively large size of the active electrode and known distant effects of the stimulation in functionally connected areas (Antal et al., 2011; Lindenberg et al., 2013; Meinzer et al., 2012), additional areas implicated with phonological retrieval and working memory (e.g., dorsal inferior frontal or parietal cortex, Hulten et al., 2010; Laine and Salmelin, 2010)) or learning (e.g., hippocampus, Breitenstein et al., 2005; Davis and Gaskell, 2009; Meinzer et al., 2010) may have been affected. This possibility needs to be explored in future complementary functional imaging studies.

Moreover, the primary goal of this proof-of-principle study was to assess whether atDCS can enhance learning and maintenance of these gains, therefore, we did not address possible detrimental effects of cathodal tDCS (Cohen Kadosh et al., 2010). However, cathodal effects on cognition are typically less consistent than in the motor domain, possibly due to greater redundancy of brain networks supporting cognition (Jacobson et al., 2012). In this context, a previous study that used a similar word learning paradigm did not show detrimental effects of cathodal tDCS on short-term phonological learning (Flöel et al., 2008).

4.3. Implications for clinical studies: Several recent studies have explored the impact of atDCS on language re-learning in chronic aphasia using different treatment paradigms and stimulation sites. However, despite overall beneficial effects, atDCS-induced gains were highly variable between patients. This might be related to the unknown relationship between language system reorganization and stimulation site which has precluded identification of the best stimulation site for individual patients (for review and additional discussion see Meinzer et al., 2011). Therefore, proof-of-principle studies in healthy groups are urgently needed to explore the extent of potential add-on effects of atDCS under highly controlled conditions. This was successfully accomplished in the present study. Please note, it has been shown that atDCS effects may even be more pronounced with advanced age (Hummel et al., 2010) and also in stroke patients. For example, while previous pilot studies in patients with post-stroke aphasia yielded mixed results, two of these studies reported that atDCS resulted in enhanced treatment outcome in the range of 20.2-38.2% (see Holland and Crinion, 2012). Similarly, a number of studies in the motor domain showed that treatment + atDCS resulted in more pronounced gains (>20%) than treatment alone (e.g., Lindenberg et al., 2010). Therefore, atDCS may be a viable tool to reduce age-associated functional decline (Meinzer et al., 2013) and to enhance treatment success in stroke patients.

Given the beneficial effects of atDCS in the present study, an important question with regard to its clinical use arises from a risk-benefit analysis. While atDCS compared to sham induced additional gains of approximately 15% during the recall task by the end of the training compared to sham, even larger gains

have been reported by previous studies that assessed the impact of dexamphetamine on novel word learning (20-35%, Whiting et al., 2007, 2008). However, due to its noradrenergic actions (Boyeson and Feeney, 1990), dexamphetamine may induce cardiovascular dysregulation (e.g., elevated systolic blood pressure and heart rate; Goldstein (1997)) which may increase mortality in stroke patients (Martinsson et al., 2003) and therefore, leave only a fraction of stroke patients eligible for such adjunct treatment. On the other hand, tDCS presents with an excellent safety profile particularly in vulnerable populations like stroke patients. In particular, so far, only mild adverse events like itching and skin irritation have been reported but no cardiovascular side effects or seizures (Brunoni et al., 2011; Floel and Cohen, 2010). In sum, given the excellent safety profile of tDCS, the present study supports further testing of atDCS as an adjunct treatment in language rehabilitation.

4.4. Limitations: A number of limitations of the present study need to be acknowledged. First, previous studies suggested that repeated stimulation impacts on protein synthesis, i.e., the neural mechanism underlying skill acquisition (Reis et al., 2009). This may explain long-term maintenance of stimulation effects over several months in previous studies that used similar stimulation protocols in other domains (Cohen Kadosh et al., 2010; Reis et al., 2009). While similar mechanisms may underlie atDCS effects in the present study, we only assessed stability of the stimulation effect for one week and future studies are indicated that employ longer follow-up periods. Second, due to the fact that we did not employ a second testing session prior to each daily training period, we could not distinguish whether online and offline effects mediate superior training gains, as demonstrated previously in the motor domain (Reis et al., 2009; Zimerman et al., 2013). Indeed, we deliberately opted against such a design, because repetition of the recognition task would have involved an additional "training" component (due to the forced choice character of the task and the expected early near ceiling effect during this task) that we wished to avoid in the present study. Third, the present behavioural proof-of-principle study did not assess the neural correlates of atDCS-induced superior learning which need to be determined in the future. In addition, we did not administer the cognitive test battery again after the end of the training. Therefore, we cannot exclude that superior learning during atDCS can partly be explained by domain general cognitive enhancement.

4.5. Conclusions: The present study provides direct evidence that atDCS facilitates language learning over repeated sessions in healthy individuals and that these gains are maintained over time. Thereby, our results contribute important novel information about the potential of atDCS to enhance language re-learning in clinical populations. In addition, while the present study employed an artificial language learning paradigm, future studies should explore whether atDCS may be useful to enhance language learning in real-world settings, e.g., in the context of foreign language acquisition.

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Figure captions

Figure 1 Study overview. (A) Shows the basic set-up of a training session. Participants were sitting in front of a notebook with tDCS-electrodes attached to the scalp. atDCS or sham were administered for 20 minutes during each daily learning session. (B) The stimulation site was located at the posterior temporo-parietal junction (red dot) that was determined using the EEG-10-20 system (yellow). (C) Illustrates the study design. Prior to study inclusion, a comprehensive neuropsychological test battery was administered. Afterwards, participants were randomly assigned to five days of training either with atDCS or sham stimulation (N=20/group). Two memory tasks were completed after the end of each daily training session. During the follow-up assessment (one week after the end of the training), only the memory tasks were assessed. (D) Illustrates a daily training and testing session. Participants were initially presented with 120 object - non-word pairs. Subsequently, the two memory tests were administered (recall and recognition tasks).

Figure 2 Shows learning curves for familiar (left panel) and novel objects (right panel) in the two stimulation groups (% correct responses, mean values and standard error of the mean are displayed) across the five learning sessions ($day_{1\text{-}5}$) and during the follow-up assessment one week later. Results are shown separately for the two memory tasks (upper row: recall task; lower row: recognition tasks). Please note: For the forced choice recognition task, results are displayed for values above chance level (50% correct). Overall, participants that received additional atDCS during learning showed more pronounced gains and the learning curves were steeper for both object types during the recall task. During the forced recognition choice task, the atDCS group performed better for novel objects and reached ceiling levels earlier for familiar objects. In addition, superior gains attained by the end of the training were maintained during the follow-up assessment.

Table 1 Demographic characteristics of the two stimulation groups (mean/standard deviation, p-values based on unpaired t-tests)

	atDCS (N = 20)		**sham (N = 20)**		
	mean	SD	mean	SD	p-value
Age (years)	24.4	3.7	23.6	3.3	0.51
Years of Education	15.7	1.4	15.9	1.6	0.65
Handedness Score	82.3	16.6	77.7	18.5	0.43
# Foreign Languages fluently spoken	1.3	0.6	1.6	0.8	0.21
BDI	3.7	2.8	3.6	3.7	0.89
Digit Span (forward)	9.2	1.8	8.7	2.1	0.44
Digit Span (backward)	8.4	2.2	8.0	2.6	0.57
Rey-Figure (copy)	35.9	0.4	35.5	1.3	0.22
Rey-Figure (delayed recall)	24.5	3.9	24.4	5.4	0.94
Phonemic Fluency (RWT, letter B)	16.4	4.3	15.7	5.0	0.62
Semantic Fluency (RWT, food)	24.6	6.7	23.7	5.1	0.63
Synonym-Antonym Test (SASKA)	45.0	5.9	43.7	5.8	0.50
Vocabulary Test (MWT)	31.3	2.7	30.1	3.8	0.26
Trail Making Test A (sec)	22.0	7.0	23.9	5.2	0.35
Trail Making Test B (sec)	44.1	14.5	45.9	11.9	0.68
Verbal Learning and Memory Test (VLMT) immediate recall (learning block 5, max. 15)	14.6	0.8	14.4	0.9	0.40
VLMT learning success (block 5 minus block 1)	6.5	2.2	6.3	2.0	0.74
VLMT (delayed free recall)	13.8	1.4	13.4	1.9	0.49

Table 2 Adverse event ratings in the two stimulation groups (mean/standard deviation; 1=none, 2=mild, 3=moderate, 4=intense, 5=very intense; p-values based on unpaired t-tests).

	atDCS		**sham**		
	mean	SD	mean	SD	p-value
Headache	1.15	0.48	1.15	0.5	0.50
Neck pain	1.00	0.00	1.25	0.08	0.08
Aching scalp	1.40	0.66	1.25	0.22	0.22
Tickling	2.30	0.84	2.00	0.17	0.17
Itching	2.45	0.97	1.80	0.03	0.03
Burning	1.70	0.9	1.60	0.36	0.36
Skin irritation	1.35	0.79	1.20	0.25	0.25
Tiredness	1.30	0.56	1.35	0.41	0.41
Loss of concentration	1.10	0.3	1.20	0.23	0.23
Mood swings	1.15	0.48	1.10	0.35	0.35

Table 3: Illustrates details of performance during the two memory tasks (raw data and % correct) for the learning period ($days_{1-5}$) and the follow-up assessment one week later. Data is displayed separately for the stimulation groups (atDCS; sham), tasks (recall; recognition) and object types (familiar; novel, N=60 items/type)

			atDCS						sham					
Object type	**Task**	**Day**	**Mean (raw)**	**SD (raw)**	**95% CI (raw)**	**Mean (%)**	**SD (%)**	**95% CI (%)**	**Mean (raw)**	**SD (raw)**	**95% CI (raw)**	**Mean (%)**	**SD (%)**	**95% CI (%)**
Familiar objects	Recall	Day 1	3.0	2.8	[1.7;4.3]	5.0	4.6	[2.9;7.1]	2.1	2.2	[1.1;3.1]	3.5	3.7	[1.8;5.2]
		Day 2	17.9	10.3	[13.1;22.7]	29.8	17.2	[21.8;37.9]	12.5	8.3	[8.6;16.3]	20.8	13.8	[14.4;27.2]
		Day 3	32.5	12.1	[26.8;38.2]	54.2	20.2	[44.7;63.6]	25.6	13.0	[19.5;31.6]	42.6	21.6	[32.5;52.7]
		Day 4	43.3	12.1	[37.6;49.0]	72.2	20.2	[62.7;81.6]	36.1	14.4	[29.3;42.8]	60.1	24.1	[48.8;71.3]
		Day 5	49.9	8.2	[46.0;53.7]	83.1	13.6	[76.7;89.4]	41.2	14.0	[34.7;47.7]	68.7	23.3	[57.8;79.6]
		Follow-up	43.0	10.8	[37.9;48.0]	71.6	18.0	[63.2;80.0]	34.7	16.2	[27.1;42.3]	57.8	27.0	[45.2;70.5]
	Recognition	Day 1	43.9	3.7	[42.2;45.7]	73.2	6.1	[70.3;76.2]	42.4	6.2	[39.3;45.4]	70.6	10.3	[65.5;75.7]
		Day 2	55.9	2.4	[54.8;57.0]	93.2	3.9	[91.3;95.0]	53.1	4.8	[50.8;55.4]	88.5	8.0	[84.6;92.3]
		Day 3	59.4	1.2	[58.9;59.9]	99.0	1.9	[98.1;99.9]	56.7	4.1	[54.8;58.6]	94.6	6.8	[91.4;97.7]
		Day 4	59.4	0.8	[59.0;59.8]	99.0	1.3	[98.4;99.6]	58.5	2.6	[57.3;59.8]	97.6	4.4	[95.5;99.6]
		Day 5	59.9	0.4	[59.7;60.0]	99.8	0.6	[99.5;100]	58.8	1.9	[58.0;59.7]	98.1	3.1	[96.6;99.5]
		Follow-up	59.6	0.8	[59.2;59.9]	99.3	1.3	[98.6;99.9]	58.1	2.5	[56.9;59.3]	96.8	4.1	[94.9;98.8]
Novel objects	Recall	Day 1	0.7	0.9	[0.3;1]	1.1	1.4	[0.4;1.7]	0.6	1.0	[0.1;1]	0.9	1.7	[0.1;1.7]
		Day 2	5.0	4.8	[2.7;7.2]	8.3	7.9	[4.5;12.0]	3.4	3.6	[1.7;5]	5.6	5.9	[2.8;8.4]
		Day 3	15.0	11.8	[9.5;20.5]	25.0	19.6	[15.8;34.2]	10.0	9.2	[5.7;14.3]	16.6	15.3	[9.4;23.8]
		Day 4	25.8	14.9	[18.8;32.7]	42.9	24.9	[31.3;54.6]	17.5	13.8	[11.0;23.9]	29.1	23.1	[18.3;39.9]

	Day 5	36.0	13.7	[29.6;42.4]	60.0	22.8	[49.4;70.6]	25.1	16.0	[17.7;32.6]	41.9	26.7	[29.4;54.4]
	Follow-up	27.1	13.7	[20.7;33.4]	45.1	22.8	[34.4;55.7]	18.5	14.9	[11.5;25.4]	30.8	24.9	[19.1;42.4]
Recognition	Day 1	36.5	3.5	[34.8;38.2]	60.8	5.8	[58.0;63.6]	35.0	5.2	[32.4;37.6]	58.4	8.7	[54.1;62.7]
	Day 2	46.0	6.8	[42.8;49.2]	76.7	11.3	[71.4;81.9]	41.5	6.7	[38.3;44.7]	69.2	11.1	[63.8;74.5]
	Day 3	53.9	4.8	[51.7;56.2]	89.9	8.0	[86.2;93.6]	48.9	6.5	[45.8;51.9]	81.5	10.9	[76.4;86.5]
	Day 4	57.0	3.3	[55.4;58.5]	95.0	5.5	[92.4;97.6]	52.8	5.5	[50.3;55.4]	88.1	9.1	[83.8;92.3]
	Day 5	58.9	2.1	[57.9;59.8]	98.1	3.5	[96.5;99.7]	55.6	4.6	[53.4;57.7]	92.7	7.7	[89.1;96.2]
	Follow-up	57.7	2.3	[56.5;58.9]	96.2	4.3	[94.1;98.2]	53.5	6.0	[50.7;56.3]	89.2	10.0	[84.5;93.9]

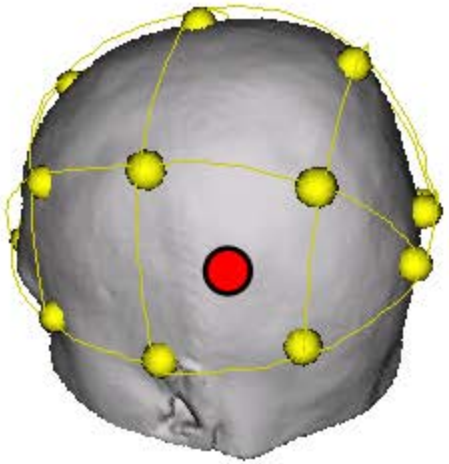
SD=standard deviation, CI=confidence intervals

Table 4 Mean and standard deviation of positive and negative mood ratings for the two stimulation groups (pooled across days)

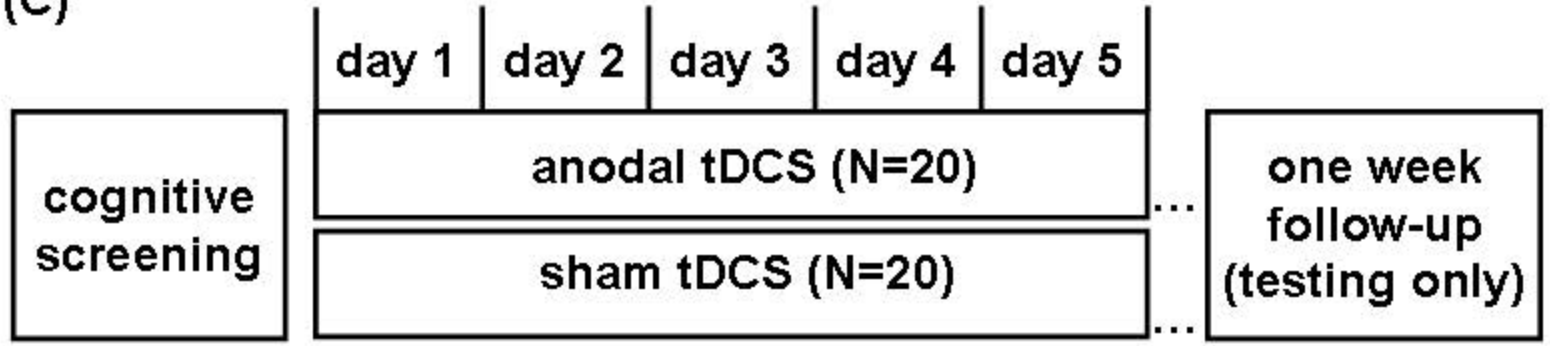
		atDCS		**sham**	
		mean	SD	mean	SD
Positive	pre	2.8	1.0	3.0	1.1
	post	3.0	1.1	3.1	1.0
Negative	pre	1.2	0.5	1.2	0.5
	post	1.1	0.5	1.2	0.5

(A)

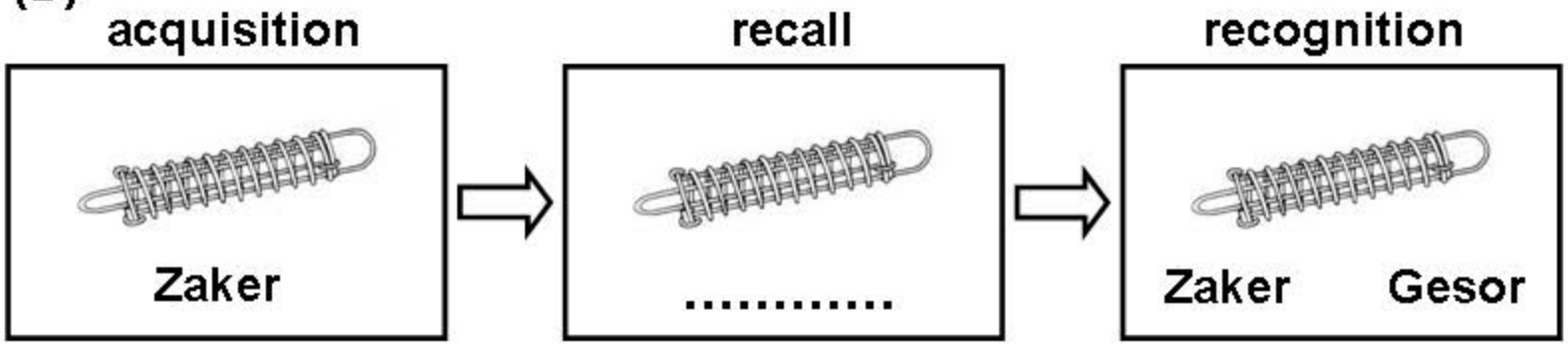
(B)

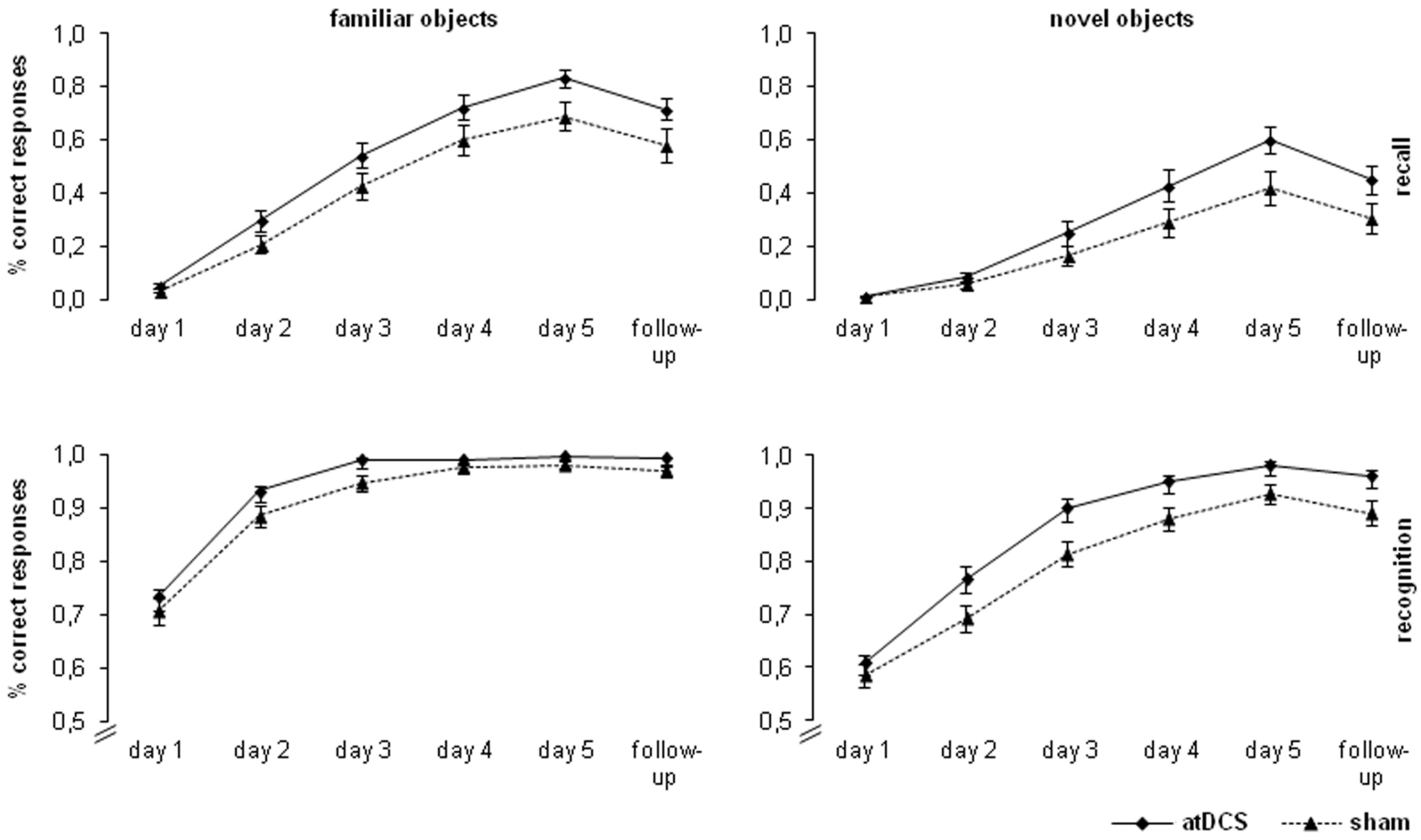


(C)


day 1
day 2
day 3
day 4
day 5
cognitive screening
anodal tDCS (N=20)
sham tDCS (N=20)
one week follow-up (testing only)

(D)


acquisition
recall
recognition
Zaker
.....
Zaker
Gesor


familiar objects
novel objects
% correct responses
recall
recognition
1,0
0,8
0,6
0,4
0,2
0,0
0,9
0,7
0,5
day 1
day 2
day 3
day 4
day 5
follow-up
atDCS
sham