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tDCS to temporoparietal cortex during familiarisation enhances the subsequent phonological coherence of nonwords in immediate serial recall

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ABSTRACT

Research has shown that direct current stimulation (tDCS) over left temporoparietal cortex – a region implicated in phonological processing – aids new word learning. The locus of this effect remains unclear since (i) experiments have not empirically separated the acquisition of phonological forms from lexical-semantic links and (ii) outcome measures have focused on learnt associations with a referent rather than phonological stability. We tested the hypothesis that left temporoparietal tDCS would strengthen the acquisition of phonological forms, even in the absence of the opportunity to acquire lexical-semantic associations. Participants were familiarised with nonwords paired with (i) photographs of concrete referents or (ii) blurred images where no clear features were visible. Nonword familiarisation proceeded under conditions of anodal tDCS and sham stimulation in different sessions. We examined the impact of these manipulations on the stability of the phonological trace in an immediate serial recall (ISR) task the following day, ensuring that any effects were due to the influence of tDCS on long-term learning and not a direct consequence of short-term changes in neural excitability. We found that only a few exposures to the phonological forms of nonwords were sufficient to enhance nonword ISR overall compared to entirely novel items. Anodal tDCS during familiarisation further enhanced the acquisition of phonological forms, producing a specific reduction in the frequency of phoneme migrations when sequences of nonwords were maintained in verbal short-term memory. More of the phonemes that were recalled were bound together as a whole correct nonword following tDCS. These data show that tDCS to left temporoparietal cortex can facilitate word learning by strengthening the acquisition of long-term phonological forms, irrespective of the availability of a concrete referent, and that the consequences of this learning can be seen beyond the learning task as strengthened phonological coherence in verbal short-term memory.

1. INTRODUCTION

A wealth of neuroimaging studies show that structures within left temporoparietal cortex (TPC), including supramarginal gyrus, posterior superior temporal gyrus and sulcus, and temporoparietal junction within the sylvian fissure, contribute to phonological processing and verbal short-term memory (STM). In posterior perisylvian cortex, activation during phonological encoding is positively associated with subsequent memory for nonwords (Clark & Wagner, 2003; see also Breitenstein et al., 2005; Paulesu et al., 2009) and for foreign words (Veroude, Norris, Shumskaya, Gullberg, & Indefrey, 2010). Left temporoparietal activity during the repetition of nonwords correlates with phonological-lexical learning and is associated with the retrieval of whole word phonology (Majerus et al., 2005; Graves, Grabowski, Mehta, & Gupta, 2008). Moreover, the left supramarginal gyrus has been specifically linked with the perception (e.g., Jacquemot, Pallier, LeBihan, Dehaene, & Dupoux, 2003; Raizada & Poldrack, 2007; Turkeltaub & Coslett, 2010; Liebenthal, Sabri, Beardsley, Mangalathu-Arumana, & Desai, 2013) and sequencing of phoneme segments (Gelfand & Bookheimer, 2003; Moser, Baker, Sanchez, Rorden, & Fridriksson, 2009).

This literature is consistent with recent findings showing that electrical stimulation to left TPC augments the learning of nonword phonological forms. Transcranial direct current stimulation (tDCS) involves passing a small current through the brain via electrodes on the scalp. Cortical excitability is increased (and the capacity for learning enhanced) by positioning the anode over a functionally-relevant site, and the cathode (or reference electrode) over a distinct scalp or extra-cephalic site (see Nitsche et al., 2008). Anodal stimulation to left TPC has been shown to boost the acquisition of new vocabulary, using both auditory and written stimuli (Flöel, Rösser, Michka, Knecht, & Breitenstein, 2008; Meinzer et al., 2014). Flöel et al. (2008) found that tDCS to TPC facilitated the learning of pairings between spoken monosyllabic nonwords and pictures of familiar

objects using a statistical learning paradigm. Fiori et al. (2008) reported that tDCS to this location aided the retrieval of newly-acquired picture names in healthy subjects and had a similar effect on picture naming in participants with aphasia. Meinzer et al. (2014) extended this work in healthy subjects by applying tDCS on five consecutive days: anodal tDCS increased the cumulative learning seen for written nonwords paired with both familiar (nameable) objects and unfamiliar entities, and these gains were still present at a follow-up one week after the last stimulation session. These findings indicate that repeated applications of tDCS can have long-term benefits on word learning. In contrast, the long-term effects of a *single* application of tDCS have not been established because these previous studies assessed the effects of tDCS immediately after stimulation, when potential effects on long-term learning are confounded by short-term increases in cortical excitability.

In addition, while these studies show that tDCS can facilitate word learning, the mechanisms underpinning these effects are not clear. In particular, further research is needed to establish whether tDCS over left temporoparietal cortex produces improvement in word learning by facilitating (i) the acquisition and retrieval of associations between new words and objects (i.e., lexical-semantic learning) or (ii) the learning of novel phonological forms themselves even in the absence of an association with a meaningful object (i.e., acquisition of phonological-lexical representations). Studies to date have exclusively examined new word learning when a concrete referent is present. However, if left temporoparietal cortex is critical for encoding phonemic sequences into new phonological-lexical representations (as indicated by, for example, Gupta & MacWhinney, 1997; Baddeley, 2003; Sato et al., 2004; Graves et al., 2008; Paulesu et al., 2009), we would anticipate that tDCS to left temporoparietal cortex would have a similar effect on nonword learning with and without the availability of a concrete referent, at least when using tasks that tap the stability of phonological processing per se as opposed to vocabulary knowledge.

To test this possibility, we made use of research showing strong effects of phonological-lexical learning on the stability of the phonological trace in immediate serial recall (ISR) tasks. In ISR, participants hear a sequence of words or nonwords and have to repeat the sequence back immediately in the correct order. The frequency with which phonemes from one item break apart and recombine with the elements of other items depends on the availability of long-term phonological-lexical representations (Jefferies, Frankish, & Lambon Ralph, 2006a, 2006b; Jefferies, Frankish, & Noble, 2009; Hoffman, Jefferies, Ehsan, Jones, & Lambon Ralph, 2009). For example, in healthy participants, we know that when the phonological system attempts to maintain several unfamiliar nonwords in order, phonological segments frequently migrate and recombine with the elements of other items or are lost altogether (for example, 'gid heem jurn' might be recalled as 'jid heen churm'). In contrast, when target items are familiar words, the frequency of these errors is greatly reduced and instead entire items are recalled out of sequence (for example, 'sash, king, cot, wall, heap' might be recalled as 'sash, cot, wall, king, heap) (Ellis, 1980; Treiman & Danis, 1988; Jefferies et al., 2006a, 2009). Such lexicality effects in ISR are preserved when words and nonwords are mixed predictably (or unpredictably) in the same list (Jefferies et al., 2006a, 2009; Hoffman et al., 2009), showing that they cannot solely reflect a strategic editing process during speech production, in which nonword responses in word lists are replaced with real words based on knowledge of the lexical status of the targets (although such effects are also observable; Jefferies et al., 2009). Instead, it appears that there is a direct impact of lexical knowledge on phonological stability. ISR data such as these provide a strong case for verbal short-term memory drawing on long-term linguistic representations (Patterson, Graham, & Hodges, 1994; Jefferies et al., 2006a; Acheson, Hamidi, Binder, & Postle, 2011), in line with a wealth of studies which show effects of lexicality (i.e., words vs. nonwords: Hulme, Maughan, & Brown, Gordon, 1991; Hulme, Roodenrys, & Brown, 1995; Saint-Aubin & Poirier, 1999, 2000; Majerus & Linden, 2003) and phonotactic

frequency (i.e., differences in phonotactic frequence between nonwords: Nimmo & Roodenrys, 2002; Majerus & Linden, 2003; Thorn & Frankish, 2005) on ISR accuracy at the whole-item level. We hypothesise that lexical learning involves acquiring a phoneme sequence, allowing the identity of upcoming phonological elements to be predicted, and that in ISR tasks, when several distinct items must be maintained simultaneously, these long-term phonological sequences are activated and place constraints on the order of phonological elements reducing phoneme migration errors. If tDCS to left TPC strengthens the acquisition of phonological-lexical representations, this stimulation should also reduce the frequency of phoneme migration errors, even when ISR is tested 24 hours later.

In order to be confident that the ISR design we developed would be sensitive to phonological familiarisation with nonwords without an influence of tDCS, we compared the recall of entirely unfamiliar nonwords with nonwords familiarised under sham conditions. This comparison establishes whether limited exposure to phonological forms 24 hours earlier is sufficient to yield better (i.e., more phonologically coherent) ISR performance. This is an important addition to the literature since previous studies comparing words and nonwords have only established a recall advantage for well-established phonological-lexical representations that have been acquired over long periods of time, and which, in the case of real words, may further benefit from independent support from those words' corresponding semantic representations (Bourassa & Besner, 1994; Patterson et al., 1994; Poirier & Saint-Aubin, 1995; Knott & Patterson, 1997; Saint-Aubin & Poirier, 1999; Walker & Hulme, 1999; Jefferies, Jones, Bateman, & Lambon Ralph, 2005; Hoffman et al., 2009).

With a (non)word learning paradigm we could test the effect on phonological maintenance of recently acquired phonological-lexical information without the confounds of additional semantic

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information provided by real words. In a study adopting a similar approach, Melby-Lervåg and

Hulme (2010) indirectly provided data indicating that simple familiarity with a phonemic sequence

may support its maintenance in verbal short-term memory. They tested children on their ISR span

for a set of low frequency unfamiliar words, delivered training on half of those words over ten days

and then re-tested ISR span. Children who received phoneme awareness training on a set of the

test items showed the greatest improvement in ISR span relative to untrained words; in contrast,

training on semantic definitions had a stronger effect on free recall. ISR span also improved for the

untrained words, presumably because the children had been exposed to the phonological forms of

these items during testing. We built on this study to test whether improvements in short-term

recall for auditory nonwords after brief phonological familiarisation occur at the phoneme level, in

terms of more stable phonological traces for familiarised nonwords.

Thus, in the present study, we examined how the stability of the phonological trace is

influenced by (i) familiarity with the phonological forms of targets, (ii) the availability of visual

associations during the learning process and (iii) the application of tDCS to left temporoparietal

cortex during learning. We considered accuracy and errors in ISR at the level of individual

phonemes – we could therefore specifically investigate whether these factors influenced the

frequency of phoneme order errors. In addition to pairing nonwords with a meaningful visual object

as in previous tDCS studies (Flöel, Rösser, Michka, Knecht, & Breitenstein, 2008; Meinzer et al.,

2014), we examined the acquisition of spoken phonological forms *without* a clear visual referent.

We contrasted two training conditions: (i) pairings of nonwords to unfamiliar concrete referents –

i.e., items that did not have a pre-existing name (as in the study by Meinzer et al., 2014) and (ii)

pairings of nonwords with blurred images that did not include specific discernable features. This

enabled us to examine whether tDCS specifically augmented the learning of word-object

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associations or instead strengthened the acquisition of a familiar phonological form (with or without a referent). We predicted that tDCS over the left temporoparietal cortex would enhance learning of the phonological forms of nonwords (even without a picture referent), and that this effect on learning would manifest in terms of a more stable phonological trace during ISR for familiarised nonwords the following day, even after short-term direct effects of tDCS on cortical excitability had dissipated.

2. METHOD

2.1. Study Overview

The study employed a within-subjects design. Participants were tested at the same time of day over three consecutive days. On Day One, they were familiarised with the first set of nonwords whilst they received sham or anodal stimulation. Some nonwords were paired with an image of a novel object (concrete referent condition), while others were paired with a blurred image (no referent condition). The blurred images did not provide any meaningful associations (see Figure 1A). On Day Two, participants performed ISR for the previously familiarised nonwords and a new set of unfamiliar nonwords. They were then familiarised with a new set of nonwords (which were again paired with clear and blurred images) using the other stimulation condition (i.e., sham if participants had received anodal stimulation on Day One, and vice versa). On Day Three, ISR was tested for the familiarised nonwords trained on Day Two with another set of unfamiliar nonwords. Stimulation order and allocation of nonword sets to conditions was fully counterbalanced across participants. We can therefore be confident that any beneficial effects of tDCS did not reflect short-term changes in neuronal excitability but instead reflected effects of phonological-lexical learning on phonological stability.

2.2. Participants

Participants were twenty-four native British English students from the University of York (aged between 18 and 29 years; 8 males), screened for contraindications for receiving tDCS. All participants were right-handed with normal hearing and normal or corrected-to-normal vision, and were paid for their participation. Two participants who did not perform ISR in serial order (i.e., who did not follow the instructions) were excluded and replaced. The study was approved by the local Research Ethics Committee.

2.3. Stimuli

2.3.1. Nonwords

120 bisyllabic spoken nonwords with a CVCVC structure were created (C = consonant, V = vowel, e.g., "vaitag" /vetæg/; further examples can be found in Figures 1 and 2). Each nonword was designed such that, across an ISR test set of 60, (i) a given consonant was present a maximum of four times in any phoneme position, (ii) there were no repetitions of CVCs and (iii) stimuli did not have a close English phonological neighbour. Stimuli were recorded by a female British English speaker with stress placed on the first syllable. The nonwords were independently rated for word-likeness (rated on a 5 point Likert scale; n=16) and scored for phonotactic probability (as per Vitevitch and Luce, 2004). The two sets of 60 stimuli were used to test ISR in different sessions, and each set was divided between three conditions (i.e., familiarised with concrete referents, familiarised with no referents and new unfamiliar). The resulting six sets of stimuli were matched for word-likeness (average per set from 2.83 to 2.98; p = .86) and phoneme and biphone position frequencies (p =.99 and p =.97 respectively). Furthermore, to ensure nonwords were phonologically distinct from each other, each nonword within its set of 20 had a different initial consonant, no vowel appeared more than twice in a given position and no V_1-V_2 combination was repeated.

Nonword stimuli were edited to one second in length, with background noise removed and average intensity controlled using Praat (www.praat.org). The allocation of each set of 20 nonwords to experimental condition was fully counterbalanced across participants (see Table 1).

For each ISR task, nonwords from the three sets (concrete referent, no referent and unfamiliar) were grouped into five lists containing four items each. Lists were created such that phonemes were not repeated for a given position within an item, allowing us to track the majority of phoneme migrations. Items were reordered twice to create 15 ISR test lists for each condition.

TABLE 1 ABOUT HERE

2.3.2. Familiarisation images

Colour images of 80 unusual objects without obvious names were selected to provide concrete referents in the familiarisation phase. These were sourced from the internet and independently rated for visual complexity and distinctiveness. They were divided into four sets of images (providing sufficient stimuli to avoid repeats across the two training sessions and the concrete vs. no referent trials). The sets were matched for complexity (average = 2.89-2.93 out of 5, p =.996) and distinctiveness (average = 2.95-2.96 out of 5, p = 1) and were randomly assigned to nonwords within their allocated condition. For the no-referent condition, a 20 pixel Gaussian blur was applied to each image using Corel PaintShop Photo Pro 3 (see Figure 1A for examples) and these images were piloted to ensure they were discriminable (i.e., participants were able to make accurate same/different judgements to pairs of these images).

2.4. Procedure

2.4.1. Transcranial Direct Current Stimulation

tDCS was applied using a direct current stimulator (DC-Stimulator Plus, NeuroConn, Germany) and saline-soaked sponge electrodes. The anode (5 × 5cm) was placed over electrode position CP5,

using the extended 10-20 system, which was used successfully in previous tDCS studies of nonword-object learning (Flöel et al., 2008; Meinzer et al., 2014). The cathode (5 × 7cm) was placed over the right supraorbital area. In the anodal condition a direct current of 1.5mA was applied for 15 minutes throughout the familiarisation task (fade in 15s, fade out 10s). In the sham condition, the current was delivered for only 30s (fade in 15s, fade out 10s).

At the end of both stimulation sessions, participants completed a tDCS sensation rating scale, which asked them to rate the duration of sensations and the intensity of itchiness, pain, burning, heat, pinching, iron taste and fatigue (adapted from Fertonani, Rosini, Cotelli, Rossini, & Miniussi, 2010). Responses showed that tDCS was well-tolerated and ratings of sensations confirmed that participants could not reliably distinguish when they had been receiving sham or active stimulation (no differences for any of the sensations, Table 2).

TABLE 2 ABOUT HERE

2.4.2. Familiarisation Task Procedure

The familiarisation procedure commenced one minute from the onset of stimulation and was identical across the two stimulation conditions. The design followed a similar principle to Flöel et al. (2008), although learning was facilitated by providing participants with feedback on the accuracy of their performance. Participants were trained to associate 40 nonwords with their respective 40 images (20 clear objects and 20 blurred images) over a total of 240 self-paced training trials. Each image was presented for 500 ms before the onset of the auditory nonword. Participants pressed one of two keys to indicate whether the pairing was correct or incorrect. This response elicited immediate visual feedback regarding the accuracy of their response. Feedback was displayed for 1s, and was immediately followed by the next trial (see Figure 1A). Presentation of stimuli was pseudo-randomised across two blocks, with two 'correct' pairings and one 'incorrect' pairing per nonword in a block. A fixed break of 15 seconds separated the two blocks. Stimuli were presented on a PC

monitor and via speakers. E-Prime (version 2.0) was used to deliver the stimuli and record responses.

FIGURE 1 ABOUT HERE

2.4.3. <u>Immediate Serial Recall Task</u>

The procedure was identical for both ISR sessions. Forty-five lists containing four nonword items were presented. Participants wore a headset with in-built microphone to listen to and recall the lists. An exclamation mark was displayed on screen from 250 ms prior to the onset of the first nonword until the offset of the fourth nonword in a list. Nonwords were presented at a rate of 1.25s per item. At the end of the presented list a question mark appeared, which acted as the cue to verbally recall the four items in serial order (see Figure 2). Participants pressed a key to indicate when they had finished recalling a list, which prompted the next trial. Participants were asked to recall items in the order in which they were presented and to attempt recall for all four items, even if unsure. They had three practice trials to familiarise themselves with the task. Verbal responses were digitally recorded.

2.5. ISR Coding

Verbal responses were phonemically transcribed phoneme-by-phoneme by two independent coders blind to the experimental conditions. When fewer than four items were produced on any given trial, whole-item omissions were positioned within the transcript in a way that minimised the error score. For example, if the participant produced three not four responses, and these largely corresponded with the second, third and fourth targets respectively, the omitted item would be positioned in the first target position (i.e., the responses would be transcribed as attempts at the second, third and fourth targets). Initial transcriptions resulted in 91% overall agreement at the phoneme level. Inconsistencies (predominantly vowels) were adjudicated by the coders for the final transcription.

Our coding scheme adapted the methods used by Jefferies et al. (2006a) to examine effects of lexicality on phonological stability. The coding scheme categorised each target phoneme as being (1) correct in position in the context of whole correct nonwords (CIP: Whole), (2) correct in position in the context of partially correct nonwords (CIP: Partial), (3) whole item order errors (ORD: Whole) – i.e., phonemes corresponding to an entire nonword produced out of sequence, and (4) phoneme migrations (MIG; as per Jefferies et al., 2006a) – i.e., target phonemes in the list that were produced in the wrong position, that did not migrate as a result of an entire *item* being produced out of sequence and had not already been captured by a CIP response. The remaining target phonemes (i.e., that were not recalled at all) were replaced by one of the following types of response: (5) repetitions (REP), in the case of incorrectly-positioned target phonemes that were produced more than once and did not correspond to a repeated production of a whole target nonword in any position (6) phoneme intrusions (INT) – i.e., responses that were not correct in position or migrated target phonemes (i.e., specifically, not CIP, MIG, ORD: Whole or REP) and (7) omissions (OM). Phoneme intrusions were identified by subtracting the number of repetitions and empty response slots (i.e., (7) the omissions) from the total target phonemes not recalled in a list.

In order for a phoneme to be classified as a MIG or REP error, the target and response phoneme were required to take the same *relative syllabic position*. Since stimuli were bisyllabic with a $C_1V_1C_2V_2C_3$ structure (where the intervocalic C_2 was typically ambisyllabic; see Anderson & Jones, 1974), migration and repetition errors could include both phonemes misplaced across the two syllables within items (e.g., from position C_1 to C_2) and phonemes misplaced across items (e.g., from the C_1 position of the first target to the C_1 or C_2 position within a different response). These migration types capture most phoneme order errors (Treiman, Straub, & Lavery, 1994).

A worked example of the coding is detailed in Figure 2.

FIGURE 2 ABOUT HERE

We reasoned that if familiarisation helps nonwords to become more word-like compared to entirely novel nonwords, more target phonemes would be retained in general in ISR, and more of these would be bound together as a whole correct nonword. Additionally, if tDCS to left TPC further supports the learning of the phonological sequences of these forms, this should lead to an additional advantage in the ordering of the phonemes (compared to sham-familiarised nonwords).

We predicted that the strengthening of phonological-lexical representations would have the following effects on ISR (within our coding scheme):

1) CIP: Whole: Increase in entirely correct items;

2) CIP: Partial: No strong prediction - these responses might decrease compared to CIP: Whole (suggesting pattern completion properties for more familiar items), or potentially increase relative to omissions and intrusions, reflecting the availability of more target phonemes;

3) ORD: Whole: These errors occur very rarely for multisyllabic nonwords and are unlikely to be sufficiently frequent to permit analysis;

4) MIG: Relative decrease, reflecting stronger phoneme binding;

5) REP: No strong prediction - some repetitions may be qualitatively similar to MIG errors, while others may replace OM errors;

6) INT: Potential decrease, reflecting the recall of more target phonemes;

7) OM: Potential decrease in missing responses, if more target phonemes are recalled.

3. RESULTS

3.1. Familiarisation Task

Familiarisation task accuracy and RT data were subjected to 2 × 2 × 4 repeated measures ANOVA (examining the factors tDCS – sham, anodal, REFERENT TYPE – referent, no referent, and

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PRESENTATION NUMBER – 1st, 2nd, 3rd and 4th repetition of the correct pairing). The task showed steady improvements in accuracy and faster reaction times as participants were exposed to more correct pairings, confirming that they were learning the associations. There were significant effects of presentation number on both accuracy [$F(3, 69) = 28.72$, $p < .001$, partial $\eta^2 = .555$] and RT [$F(3, 69) = 47.32$, $p < .001$, partial $\eta^2 = .673$]; Figure 1C. Performance was poorer overall for the no referent than the clear referent condition [main effect of REFERENT, accuracy, $F(1, 23) = 26.63$, $p < .001$, partial $\eta^2 = .537$; and RT, $F(1, 23) = 22.15$, $p < .001$, partial $\eta^2 = .491$]. There were no effects of tDCS or significant interactions for the familiarisation task.

3.2. Immediate Serial Recall (ISR)

We first examined whether the exposure to the phonological forms of nonwords provided by the familiarisation task was sufficient to improve ISR for these items the following day, even without an influence of tDCS (i.e., in the sham condition). Paired t-tests were used to compare the recall of FAMILIARISED nonwords (i.e., an average of nonwords from the clear referent vs. no referent conditions) vs. NEW nonwords from the sham condition. These data are shown in Table 3.

Secondly, we considered whether tDCS during familiarisation was able to modulate these learning effects. Effects of stimulation on ISR for familiarised words were tested in a 2 × 2 repeated measures ANOVA, which included the factors of tDCS (SHAM vs. ANODAL) and referent type (REFERENT vs. NO REFERENT). Since tDCS was applied during the initial familiarisation phase and therefore could not affect ISR performance for entirely NEW nonwords, only familiarised nonwords were included in this analysis. These data are shown in Table 4.

For both of these research questions, we conducted two analyses: one examining the frequencies of each response category as a percentage of total target phonemes presented

(identified as "Analysis 1") and another specifically examining the production of target phonemes as a percentage of the number of phoneme targets recalled in any position (i.e., the sum of CIP: Whole, CIP: Partial, ORD: Whole, MIG, REP; not performed for non-target responses). This analysis, identified as "Analysis 2", removes the influence of unrelated intrusions and omissions on overall performance and examines the stability of target phoneme recall, i.e., how many phonemes were successfully bound together as items, and how many split apart and migrated separately (see Jefferies et al., 2006a, for use of a similar approach).

Analyses of the total phonemes recalled in any position (i.e., the baseline used to produce percentages in Analyses 2) revealed greater recall of target phonemes for familiarised words [72% of NEW targets vs. 78% of familiarised targets; $t(23) = 3.66$, $p = .001$, $d = -.51$, but the total number of target phonemes produced was not changed by tDCS [78% of both sham targets and of tDCS targets, $F(1, 23) = 0.37$, ns] or referent condition [all $p > .85$]. This indicates that familiarisation helped participants to retain more phonemes compared to completely novel nonwords, but tDCS at the time of familiarisation did not produce any benefit in overall recall capacity compared to sham. The relevant data for each response category is summarised in Table 5.

TABLES 3, 4 and 5 ABOUT HERE

3.2.1. Phonemes correct-in-position forming whole nonwords (CIP: Whole)

3.2.1.1.   Analysis 1 (percentage of phonemes presented)

On average, approximately 26% of the target phonemes were recalled as part of whole correct nonwords. Phonemes from familiarised nonwords were more frequently recalled than phonemes from NEW nonwords [effect of FAMILIARITY, $t(23) = 6.72$, $p < .001$, $d = .765$; Table 3].

For nonwords that were previously familiarised, there was a non-significant trend for more phonemes to be correct in position as part of whole nonwords when familiarisation had proceeded

under tDCS conditions compared to under sham [sham mean: 28%, tDCS mean: 32%; *p* = .060, partial η^2 = .146; Table 4 and Figure 3; see next paragraph for further analyses]. Referent type during familiarisation had no effect [all *p* > .14].

3.2.1.2. Analysis 2 (percentage of target phonemes recalled in any position)

In the second analysis, however, whole nonword recall was found to be significantly enhanced by both familiarisation [effect of FAMILIARITY, *t*(23) = 6.96, *p* < .001, *d* = - .828; Table 5] and by tDCS at familiarisation [effect of tDCS, *F*(1, 23) = 5.81, *p* = .024, partial η^2 = .202], relative to new nonwords and sham-familiarised nonwords respectively, suggesting stronger pattern completion properties under these conditions. No other modulation of CIP: Whole was observed [all *p* > .13].

To confirm that this tDCS-related increase in phonemes recalled as part of a whole nonword did not reflect a general difference across testing sessions, the rate of respective phonemes out of position elicited by the NEW nonwords in both ISR tasks was also compared. There was no difference in the rates of correctly recalled whole unfamiliar nonwords between sessions [sham session NEW vs. tDCS session NEW CIP: Whole: *t*(23) = .01, *ns*].

3.2.2. *Phonemes correct-in-position forming partially correct nonwords (CIP: Partial)*

3.2.2.1. Analysis 1 (percentage of phonemes presented)

CIP: Partial responses accounted for 28% of target phonemes. The overall frequency of these phoneme responses was not modulated by any of the experimental conditions [all *p* > .16].

3.2.2.2. Analysis 2 (percentage of target phonemes recalled in any position)

In the second analysis, the rates of phonemes corresponding to partially correct nonwords were significantly *reduced* for familiarised nonwords compared to new nonwords [effect of FAMILIARITY, *t*(23) = -3.31, *p* = .003, *d* = - .655; Table 4]. No other modulation of CIP: Partial phoneme rates were observed [all *p* > .16].

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3.2.3. Item Order Errors (ORD: Whole)

These occurred extremely rarely (under 2% of target items, with the majority of these errors produced by a few participants). Due to their low frequency these were not analysed further (see Tables, 3, 4, and 5 for their respective frequencies).

Phoneme Migrations (MIG)

3.2.3.1. Analysis 1 (percentage of phonemes presented)

The majority of incorrect responses were target phonemes recalled out of sequence (21% when including repetitions) and a little over half of these were phoneme migrations (over 11% of targets). The number of phoneme migrations was influenced by prior familiarisation [MIG effect of FAMILIARITY, $t(23)$ = -2.76, p = .011, d = .384; Table 3], indicating that minimal exposure to the phonological forms of the nonwords had a lasting influence on the stability of the phonological trace the following day.

Fewer phoneme migrations occurred for those nonwords familiarised during the application of anodal tDCS compared to sham [main effect of tDCS: $F(1,23)$ = 9.53, p = .005, partial η^2 = .293; Table 4 and Figure 3]. There was, however, no effects of referent type overall [$F(1,23)$ = 1.71, ns] and tDCS during familiarisation did not interact with referent type [$F(1,23)$ = 0.86, ns].

To confirm that this tDCS-related reduction in phoneme movement was specific to the effects of training with tDCS and did not reflect a general difference across testing sessions, the number of migrated phonemes elicited by the NEW nonwords in both ISR tasks was compared. There was no difference in phoneme migrations for unfamiliar nonwords between sessions [sham session NEW vs. tDCS session NEW MIG: Whole: $t(23)$ = -0.24, ns].

3.2.3.2.   Analysis 2 (percentage of target phonemes recalled in any position)

A similar pattern of results were found as for overall frequencies: Rates of phoneme migrations were reduced for familiarised nonwords [effect of FAMILIARITY, t (23) = -3.99, p = .001, d = .519]. Moreover, tDCS-familiarised nonwords led to fewer incorrectly positioned target phoneme responses [effect of tDCS, $F(1,23)$ = 7.32, p = .013, partial η^2 = .242]. No other modulation of phoneme migration rates was observed [all p > .20].

3.2.4.   *Phoneme Repetitions (REP)*

3.2.4.1.   Analysis 1 (percentage of phonemes presented)

Approximately 9% of target phonemes were produced more than once. These errors followed similar patterns to migrations but frequencies were not significantly reduced by familiarisation [$t(23)$ = -1.49, *ns*].

A reduction in the number of phoneme repetitions in ISR following tDCS was also not significant [$F(1, 23)$ = 2.65, *ns*] and there were no effects of referent type on their frequency [all p > .17].

3.2.4.2.   Analysis 2 (percentage of target phonemes recalled in any position)

When rates of phoneme repetition errors were considered in relation to target phonemes recalled in any position, their decrease for familiarised compared to new nonwords reached significance [effect of FAMILIARITY, t (23) = -3.16, p =.004, d = .401]. The influence of tDCS, on the other hand, was non-significant [$F(1,23)$ = 2.36, *ns;* Table 5], and there was no influence of referent type [all p > .19].

3.2.5. *Phoneme Intrusions (INT)*

3.2.5.1.   Analysis 1 (percentage of phonemes presented)

The intrusion of non-target phonemes accounted for 15% of target phonemes. These errors were

influenced by prior familiarisation, with more intrusions for new nonwords [effect of FAMILIARITY,

$t(23) = -3.02$, $p = .006$, $d = .262$; Table 3].

        tDCS did not modulate the production of phoneme intrusions in ISR [all $p > .29$] and there

were no effects of referent type on the frequency of phoneme intrusions.

        Analysis 2 does not apply for non-target responses.

3.2.6. *Phoneme omissions (OM)*

3.2.6.1.   Analysis 1 (percentage of phonemes presented)

Phoneme omissions accounted for approximately 8% of target phonemes. These errors were

influenced by prior familiarisation, with more omissions for new nonwords [effect of FAMILIARITY,

$t(23) = -2.26$, $p = .034$, $d = .320$; Table 3].

        tDCS did not modulate the number of omitted phonemes in ISR and there were no effects of

referent type on their frequency [all $p > .14$].

FIGURE 3 ABOUT HERE

4. DISCUSSION

This study tested whether anodal tDCS applied to left temporoparietal cortex (left TPC) during

exposure to auditory nonword forms strengthened the phonological-lexical acquisition of those

forms independently of their association with a referent image. We employed a novel verbal short-

term serial recall paradigm in which the effects of stimulation on the phonological stability of

nonwords could be assessed outside the learning and stimulation context. Our rationale was that differences in immediate serial recall performance at the phoneme level would index any tDCS-induced differences in the phonological integrity of the nonword representations. Observing such effects the day after familiarisation demonstrates that tDCS modulated the strength of long-term learning and that its effects cannot be attributed to the direct short-term physiological effects of the stimulation.

As expected, familiarised nonwords showed an overall advantage in serial recall accuracy compared to unfamiliar nonwords, related to fewer phonemes produced as part of partially correct nonwords, phoneme migrations, intrusions and omission errors for familiar items. There was a lexical-*like* advantage for the familiarised nonwords such that more of their phonemes were able to be retained, their constituent phonemes were less likely to break away from each other, and they were more likely to be recalled together as a whole unit. Since the linguistic characteristics of the nonword sets were controlled (e.g., in terms of phonotactic frequency, number of phonological neighbours, plus the frequency with which particular phonemes occurred within the stimuli) and the allocation of these sets to each experimental condition was counterbalanced between participants, we can be confident that the highly reliable difference in ISR between familiarised and unfamiliar nonwords reflected the opportunity to learn their phonological forms. This indicates that verbal STM receives substantial support from long-term information about phonological sequences that occur within the language, even when this information is newly acquired on the basis of on a few presentations (for similar conclusions from single item repetition see Majerus, Linden, Mulder, Meulemans, & Peters, 2004).

Critically we showed that the improvements in phonological maintenance for familiarised stimuli in ISR were strengthened following one fifteen-minute application of anodal tDCS to left

TPC, even in the absence of participants' awareness of the stimulated condition, and even when there was no opportunity to acquire a binding between a nonword and an object. Anodal stimulation specifically increased the stability of the phoneme sequence corresponding to trained nonwords the following day. There was a clear reduction in the number of phoneme migrations in ISR in both analyses, which was reflected in an increased tendency to recall whole nonwords in position when the familiarisation phase was accompanied by tDCS. We predicted this pattern of effects since the brain region we stimulated is associated with phoneme ordering and phonological learning (Gelfand & Bookheimer, 2003; Cornelissen et al., 2004; Majerus et al., 2005; Graves et al., 2008; Moser et al., 2009).

These data accord well with theories that propose that the short-term phonological store draws on ongoing processing within the phonological system (Patterson et al., 1994; Acheson et al., 2011; Majerus, 2013). In the familiarisation phase, the phonological system was able to partially learn the phoneme sequences corresponding to familiar nonwords: this learning gave rise to pattern completion properties, allowing the phonological system to 'anticipate' (or partially activate) phonemes that were grouped together as an item subsequently in ISR. When compared to entirely new phonological sequences, which lacked the precedent for such anticipation and grouping of their phonemes within the phonological system, verbal STM capacity for familiarised forms were enhanced overall. In a similar way, we would hypothesise that the application of left TPC stimulation during familiarisation boosted the phonological system's engagement with encoding the phonological sequences, resulting in more stable phonological representations, which would in turn have acted as a stronger source of constraint on the maintenance and recall of those phonological sequences in ISR relative to sequences familiarised without stimulation.

The ISR results demonstrate that even a single-shot application of tDCS can improve the phonological stability on nonwords the following day. We can rule out the possibility that tDCS enhanced ISR *directly*, e.g., by increasing the excitability of the phonological system *during the ISR task itself*, since the effects were observed the day after stimulation. In other words, ISR was tested long after the increases in excitability driven directly by tDCS had passed (short single doses of anodal tDCS have, so far, been shown to alter motor cortex excitability for 1-2 hours, e.g., Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; see Nitsche et al., 2008). Instead, the increased excitability elicited by anodal tDCS (during stimulation; Stagg & Nitsche, 2011) facilitated the *acquisition* of the phonological forms of the nonwords in long-term memory. This effect is likely to reflect the contributions of a number of neighbouring brain regions supporting aspects of phonological processing, including phonological short-term memory (Warrington & Shallice, 1969; Paulesu, Frith, & Frackowiak, 1993; Jonides et al., 1998; Henson, Burgess, & Frith, 2000; Buchsbaum & Esposito, 2008; Buchsbaum, Padmanabhan, & Berman, 2010; Acheson et al., 2011; Koenigs et al., 2011), phoneme sequencing (Gelfand & Bookheimer, 2003; Moser et al., 2009), translation of auditory to articulatory representations (Hickok & Poeppel, 2000; Papoutsi et al., 2009; Hickok, Houde, & Rong, 2011; Peschke, Ziegler, Eisenberger, & Baumgaertner, 2012), stimulus-driven attention (Downar, Crawley, Mikulis, & Davis, 2001; Ravizza, Hazeltine, Ruiz, & Zhu, 2011; Cabeza, Ciaramelli, & Moscovitch, 2012) and auditory processing for speech (posterior superior temporal cortex).

Since our objective was to examine effects of tDCS on later ISR, the familiarisation task was adapted to ensure nonword-image pairings were rapidly acquired with a high degree of accuracy. Our familiarisation paradigm dispensed with the errorless incidental learning element of Flöel et al. (2008) in order to accommodate a larger stimulus set and thus fewer presentations of each within

the stimulation period. The use of trial-by-trial feedback to assist nonword-image pairing, in the context of fewer trials to measure learning, will have restricted the opportunity to observe tDCS effects within the familiarisation task itself.

During familiarisation participants learned to associate clear referent images to nonwords faster than the blurred referent images, presumably because the latter were more confusable; yet ISR performance did not show a clear effect of referent type and referent type did not interact with tDCS: the application of anodal tDCS augmented both referent conditions similarly. This finding shows that tDCS to left TPC, even in the absence of a concrete referent, can enhance phonological-lexical learning. The results leave open the issue of whether effects of the referent type would be seen in ISR following a different learning procedure in which semantic content was maximised, and whether this effect would be enhanced by tDCS to a different location, such as the anterior temporal lobes or left inferior frontal region (where tDCS has previously been shown to facilitate lexico-semantic fluency and picture naming: Cattaneo, Pisoni, & Papagno, 2011; Holland et al., 2011). These questions are the focus of ongoing investigations in our lab.

Our findings help to elucidate what is being learned when tDCS to left TPC has durable effects on new word learning. In the study by Meinzer et al. (2014), tDCS enhanced cumulative learning of written nonword-image pairs over five sessions, plus naming and recognition at the end of the study. This improvement might reflect better learning of the nonwords' phonological (and orthographic) forms independently of any image. Our conclusion – that left TPC stimulation during nonword-object learning facilitates long-term acquisition of phonological-lexical forms independently of a (visual) association – is compatible with a recent fMRI study. Takashima, Bakker, van Hell, Janzen, & McQueen (2014) employed a nonword training paradigm in which nonwords were either associated with a picture of a novel object or were not associated with an image, and

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examined the BOLD response to these (and other) items immediately after training and 24 hours later. Greater left TPC recruitment immediately after training was associated with enhanced behavioural effects of lexical competition the next day and this effect was not enhanced by the availability of a visual referent.

4.1. Conclusions

tDCS during the encoding of a novel phonological form can boost the form's subsequent accuracy in an auditory-verbal short-term memory task, resulting in fewer phoneme migrations and more coherent recall of those items. We suggest that increasing temporoparietal excitability during phonological familiarisation aids the acquisition of a phoneme sequence, which constitutes phonological-lexical knowledge.

These findings indicate a likely benefit of tDCS to left TPC for tasks that emphasise phonological-lexical processing. Thus, such stimulation might prove useful in applied phonological training contexts, e.g., in concert with dyslexia interventions and new language learning. Alternative stimulation sites might better serve the binding of a phonological-lexical form with meaning.

5. ACKNOWLEDGMENTS

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Figure Captions

Figure 1. Familiarisation task procedure and performance during sham and active tDCS. (A). Illustrative trial sequence within the familiarisation task. (B.) Schematic of the anode position according to the extended 10-20 system. (C.) Improvements in accuracy and correct reaction times with each presentation of correct nonword-image pairs over the familiarisation phase.   Note that during familiarisation the first correct paired presentations of a nonword with its image were sometimes preceded by an incorrect image pairing, resulting in average above chance performance for first presentations of correctly paired stimuli.

Figure 2. An illustrative ISR trial. (A) The visual display during ISR. (B) An example target nonword list on the left and, on the right, an example spoken response. In this example, at transcription the omitted item response would be positioned in the second response slot (corresponding to the target 'leerwize') and the second and third responses would be shifted to the third and fourth response slots respectively to maximise the number of phonemes correct in position. This would code *all* phonemes for the first target as CIP as part of a whole correct item (totalling 5 CIP: Whole), two CIP phonemes in the third slot, corresponding to C_2 and V_2 of Target 3, and three in the fourth slot, corresponding to C_1, C_2,V_2 of Target 4 (totalling 5 CIP: Partial). The C_2 of Target 2 ('w') moved to C_1 of response 3, and the C_3 of Target 3 ('th') moved to C_3 of Target 4 would both be identified as phoneme migrations (2 MIG), while the V_2 of Target 1 repeated in response 4 ('ai') would be identified as a repetition (1 REP). This leaves seven non-target phoneme errors out of 20 target phonemes. The five missing phonemes corresponding to the whole missing item response and the one missing C_3 response from slot 3 would be categorised as phoneme omissions (a sum of 6 OM)

and subtracted from the non-target errors to provide the total phoneme intrusions for the list (1 INT; corresponding to the unrelated V_1 ('i') in slot 3).

Figure 3. ISR data at the phoneme level for sham- and tDCS-familiarised nonwords as percentages of target phonemes recalled in any position. The graph shows a significant increase in whole nonwords recalled correct in position and a reduction of phoneme order errors in ISR for familiarised nonwords presented under tDCS conditions relative to sham. CIP: Whole = correct in position phoneme in the context of a whole correct nonword; CIP: Partial = correct in position phoneme in the context of a partially correct nonword; ORD = Whole item order error; MIG = phoneme migration error; REP = phoneme repetition error. * p < .05.

Table 1. *An example of how nonwords were counterbalanced across familiarisation conditions.*

	Stimulus Sets					
	Familiarisation Day 1			**Familiarisation Day 2**		
Example	**Set A** "litchoit"	**Set B** "kairbung"	**Set C** "hoyroat"	**Set D** "jisharm"	**Set E** "seegark"	**Set F** "mepposh"
Set 1	Ref.: Sham	NRef: Sham	NP	Ref.: tDCS	NRef: tDCS	NP
Set 2	NP	Ref.: Sham	NRef: Sham	NP	Ref.: tDCS	NRef: tDCS
Set 3	NRef: Sham	NP	Ref.: Sham	NRef: tDCS	NP	Ref.: tDCS
Set 4	Ref.: tDCS	NRef: tDCS	NP	Ref.: Sham	NRef: Sham	NP
Set 5	NP	Ref.: tDCS	NRef: tDCS	NP	Ref.: Sham	NRef: Sham
Set 6	NRef: tDCS	NP	Ref.: tDCS	NRef: Sham	NP	Ref.: Sham

Note. Participants were allocated to one of 12 counterbalanced conditions. Six were as shown above; the remaining six followed the above structure however the 'Day 2' stimuli were delivered on Day 1, and 'Day 1' stimuli were delivered on Day 2.

Ref.: clear image association, NRef: blurred image association, NP: Not Presented (i.e., 'new' nonwords for ISR).

Table 2. *Mean tDCS sensation ratings following sham and active tDCS*

tDCS Sensations	Sham tDCS Rating		Active tDCS Rating			
	M	*SD*	*M*	*SD*	*Z*	*p*
Sensations began	0.08	0.28	0.13	0.34	-.58	.564
Duration	0.83	0.87	0.96	0.86	-.58	.559
Effect on task	0.54	0.78	0.54	0.93	.00	1.000
Itchiness	0.63	0.58	1.13	1.42	-1.81	.070
Pain	0.38	0.58	0.38	0.71	.00	1.000
Burning	0.67	0.77	0.63	0.77	-.38	.705
Warmth	0.00	0.79	0.96	0.75	-.38	.705
Pinching	0.46	0.98	0.42	0.65	-.63	.527
Iron Taste	0.13	0.61	0.00	0.00	-1.00	.317
Fatigue	0.42	0.88	0.17	0.48	-1.93	.053

Note. Sensations were rated with Likert scales. Ratings of the start and length of sensations were rated on a three-point scale (when the sensations began: 0= 'at the beginning of stimulation', 1= 'in the middle', 2= 'towards the end'; how long they lasted: 0= 'they stopped soon', 1= 'they lasted some minutes', 2= 'they lasted until the stimulation ended'). As per Fertonani et al. (2010), sensation intensities were rated on the following 5-point scale: 0=none, 1=mild, 2=moderate, 3=considerable, 4=strong. Two-tailed *p* values were obtained with Wilcoxon matched pairs tests.

Table 3. *Effects of familiarity in the absence of tDCS*

	Familiarity				
	New		Trained		*p*
	M	*SD*	*M*	*SD*	
CIP: Whole	18.06	12.49	28.23	14.61	**<.001**
CIP: Partial	29.14	8.14	27.62	7.35	.174
Item Order Errors[a]	1.25	3.08	1.94	3.48	-
Phoneme Migrations	13.44	6.84	11.05	5.57	**.011**
Phoneme Repetitions	10.35	3.84	9.48	3.78	.150
Phoneme Intrusion	17.22	9.32	15.06	7.49	**.006**
Phoneme Omission	10.54	15.14	6.62	9.12	**.033**

Note. Responses coded at the phoneme level, expressed as a percentage of targets.

Data from sham sessions only to isolate the effects of familiarity from effects of tDCS. *p*

values relate to paired comparisons, where performed (described in the text) and are

highlighted in bold where statistically significant. The rates of phoneme migrations and

repetitions comprising incorrect positioned phonemes are shown in italics. [a] Phonemes

corresponding to whole item order errors (ORD: Whole) were not analysed and are shown

for completeness only.

Table 4. *Comparison of ISR responses for nonwords familiarised in sham and anodal tDCS conditions (% of target phonemes)*

	Sham-familiarised				tDCS-familiarised				tDCS Effect	tDCS × Ref
	Sham no referent		Sham with referent		Anodal no referent		Anodal with referent			
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*p*	*p*
CIP: Whole	26.88	15.55	29.58	16.01	30.76	15.35	32.85	18.27	.060*	.853
CIP: Partial	28.61	8.15	26.63	8.03	27.40	7.00	26.08	7.97	.480	.743
Item Order Errors[a]	1.60	3.09	2.29	4.16	1.04	1.18	1.25	1.79	-	-
Phoneme Migrations	11.72	7.00	10.37	4.87	9.47	4.99	9.33	6.16	**.005**	.363
Repetitions	9.65	4.33	9.31	3.75	9.07	3.47	8.28	4.54	.117	.610
Phoneme Intrusion	15.31	7.61	14.81	7.75	14.86	8.35	13.86	8.12	.285	.628
Phoneme Omission	6.24	9.10	7.01	9.79	7.39	8.99	8.35	11.98	.140	.905

Note. All responses coded at the phoneme level, expressed as a percentage of targets. *p* values relate to repeated measures ANOVAs, where performed (described in the text) and are highlighted in bold where statistically significant. The rates of phoneme migrations and repetitions comprising incorrect positioned phonemes are shown in italics. [a] Phonemes corresponding to whole item order errors were not analysed and are shown for completeness only. * comparison was approaching significance.

Table 5. *Effects of familiarisation and tDCS on phonologically related recall in ISR (% of phonemes recalled in any position).*

	New		Sham-familiarised				tDCS-familiarised				Fam Effect	tDCS Effect
			Sham no referent		Sham with referent		Anodal no referent		Anodal with referent			
	M	SD	M	SD	M	SD	M	SD	M	SD	p	p
CIP:Whole	23.81	12.91	33.20	16.09	36.91	16.96	38.69	15.98	41.58	20.79	**<.001**	**.024**
CIP: Partial	40.41	8.43	36.35	9.01	34.10	9.02	35.36	8.03	33.49	9.25	**.003†**	.613
ORD: Whole[a]	2.00	5.67	2.27	4.68	3.13	6.00	1.36	1.52	1.72	2.46	-	-
MIG	19.45	10.37	15.68	10.10	13.72	7.10	12.69	7.63	12.46	8.71	**.001**	**.013**
REP	14.32	4.97	12.51	5.96	12.14	5.13	11.89	4.87	10.76	6.02	**.004**	.138

Note. Responses coded at the phoneme level, expressed as a percentage of target phonemes recalled in any (relative syllable) position. *p* values for the familiarisation effect relate to paired comparisons of the new nonwords and combined sham-familiarised nonwords, while the tDCS *p* values relate to main effects of the repeated measures ANOVAs (as described in the text). These are highlighted in bold where statistically significant. Fam: Familiarity. [a] Phonemes corresponding to whole item order errors were not analysed and are shown for completeness only. †Note that, unlike the effect for the CIP/Whole phonemes, this effect relates to a decrease with familiarisation.

A.

lɪtʃɔɪt
"litchoit"

Correct! :)

gɔːrpæʃ
"gorpash"

Incorrect : (

keəʳbʌŋ
"kairbung"

Correct! :)

tʃʌreək
"churrairk"

B.

C.

Legend: Sham Referent, Sham Blur, tDCS Referent, tDCS Blur

Mean Accuracy (%)

Mean Correct RT (ms)

Correct Image-Nonword presentation

1st 2nd 3rd 4th