Journal of Visualized Experiments

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--Manuscript Draft--

Article Type:	Invited Methods Article - Author Produced Video
Manuscript Number:	JoVE59159R2
Full Title:	Transcranial direct current stimulation (tDCS) of Wernicke's and Broca's areas in studies of language learning and word acquisition
Keywords:	transcranial direct current stimulation (tDCS); language; learning (acquisition); cortex; Broca; Wernicke
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$1200)

TITLE:

Transcranial Direct Current Stimulation (tDCS) of Wernicke's and Broca's Areas in Studies of Language Learning and Word Acquisition

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KEYWORDS:

tDCS, Wernicke's area, Broca's area, speech, word acquisition, language

SUMMARY:

Here, we describe a protocol for using transcranial direct current stimulation for psycho- and neurolinguistic experiments aimed at studying, in a naturalistic yet fully controlled way, the role of cortical areas of the human brain in word learning, and a comprehensive set of behavioral procedures for assessing the outcomes.

ABSTRACT:

Language is a highly important yet poorly understood function of the human brain. While studies of brain activation patterns during language comprehension are abundant, what is often critically missing is causal evidence of brain areas' involvement in a particular linguistic function, not least due to the unique human nature of this ability and a shortage of neurophysiological tools to study causal relationships in the human brain noninvasively. Recent years have seen a rapid rise in the use of transcranial direct current stimulation (tDCS) of the human brain, an easy, inexpensive and safe noninvasive technique that can modulate the state of the stimulated brain area (putatively by shifting excitation/inhibition thresholds), enabling a study of its particular contribution to specific functions. While mostly focusing on motor control, the use of tDCS is becoming more widespread in both basic and clinical research on higher cognitive functions, language included, but the procedures for its application remain variable. Here, we describe the use of tDCS in a

psycholinguistic word-learning experiment. We present the techniques and procedures for application of cathodal and anodal stimulation of core language areas of Broca and Wernicke in the left hemisphere of the human brain, describe the procedures of creating balanced sets of psycholinguistic stimuli, a controlled yet naturalistic learning regime, and a comprehensive set of techniques to assess the learning outcomes and tDCS effects. As an example of tDCS application, we show that cathodal stimulation of Wernicke's area prior to a learning session can impact on word learning efficiency. This impact is both present immediately after learning and, importantly, preserved over longer time after the physical effects of stimulation wear off, suggesting that tDCS can have long-term influence on linguistic storage and representations in the human brain.

INTRODUCTION:

The neurobiological mechanisms of human language function are still poorly understood. As the bedrock of our communication ability, this unique human neurocognitive trait plays a particularly important role in our personal and socio-economic lives. Any deficits affecting speech and language are devastating for the sufferers and expensive for the society. At the same time, in the clinic, procedures for treatment of speech deficits (such as aphasia) remain suboptimal, not least due to poor understanding of the neurobiological mechanisms involved[1]. In research, the recent advent and rapid development of neuroimaging methods have led to multiple discoveries describing activation patterns; yet, causal evidence is often still lacking. Furthermore, language areas of the brain are located somewhat suboptimally for application of mainstream neurostimulation approaches which can provide causal evidence, most importantly the transcranial magnetic stimulation technique (TMS). Whereas offline TMS protocol, such as theta burst stimulation, can cause pain due to the close proximity of the muscles to the point of stimulation, "online" TMS protocols can introduce sound artifacts from stimulation, which is undesirable as it interferes with linguistic stimulus presentation[2]. Even though TMS is widely used in language studies despite such inconveniences, a welcome alternative may be provided by other stimulation methods, most notably transcranial direct-current stimulation (tDCS). In recent years, tDCS has seen a remarkable growth in its use due to its accessibility, ease of use, relative safety and often rather striking outcomes[3]. Even though the exact mechanisms underpinning tDCS influence on neural activity are not understood completely, the mainstream view is that, at least at low intensity levels (typically 1–2 mA for 15–60 min), it does not cause any neural excitation or inhibition per se, but instead modulates the resting transmembrane potential in a graded way towards de- or hyperpolarization, shifting the excitation thresholds up or down and thereby making the neural system more or less susceptible to modulations by other events, stimuli, states or behaviors[4,5]. Whereas most of the applications reported to date have focused on the motor function[6] and/or motor system deficits, it has been increasingly applied to higher-level cognitive functions and their respective disabilities. There has been a rise in its application to speech and language, mostly in research aimed at the recovery of post-stroke aphasia[7–9], even though it has so far led to mixed results with respect to the therapeutic potential, stimulation sites and hemispheres, and optimal current polarity. As this research, and particularly the application of tDCS in cognitive neurobiology of normal language function, is still in its infancy, it is crucial to delineate procedures for stimulating at least the core language cortices (most importantly Wernicke's and Broca's areas) using tDCS, which is one the main aims of the current report.

Here, we will consider application of tDCS to language areas in a word-learning experiment. In general, the case of word learning is taken here as one example of a neurolinguistic experiment, and the tDCS part of the procedure should not change substantially for other types of language experiments targeting the same areas. Yet, we use this opportunity to also highlight major methodological considerations in a word acquisition experiment per se, which is the second main aim of the current protocol description. Brain mechanisms underpinning word acquisition—a ubiquitous human capacity at the core of our linguistic communication skill—remain largely unknown[10]. Complicating the picture, existing literature differs widely in how experimental protocols promote word acquisition, in control over stimulation parameters, and in tasks used to assess learning outcomes (see, e.g., Davis et al.[11]). Below, we describe a protocol that uses highly controlled stimuli and presentation mode, while ensuring a naturalistic context-driven acquisition of novel vocabulary. Furthermore, we use a comprehensive battery of tasks to assess the outcomes behaviorally at different levels, both immediately after learning and following an overnight consolidation stage. This is combined with sham and cathodal tDCS of language areas (we make a particular example using Wernicke's area stimulation) which can provide causal evidence on underlying neural processes and mechanisms.

PROTOCOL:

All procedures were approved by the local research ethics committee of the St. Petersburg State University, St. Petersburg, with consent obtained from all participants.

NOTE: All participants must sign the informed consent and fill in a questionnaire to attest the absence of any contraindications for tDCS stimulation (see Technique and Considerations in the Use of 4 x 1 Ring High-definition Transcranial Direct Current Stimulation (HD-tDCS) by Willamar[12]) and to collect other data relevant to the study such as vision acuity, demographics, language experience and handedness. For the latter, the seminal work by Oldfield[13] is recommended.

1. Subjects and experimental environment

1.1. In a typical language experiment, ensure that all subjects are right-handed and have no record of language deficits, neurological or psychiatric disorders. Their native language must be controlled.

1.2. Conduct all measurements in a sound-proof or at least sound-attenuated chamber. Sound insulation is very important, since any extraneous sound, noise, human speech, etc. can significantly affect the performance and thus influence the data (**Figure 1**).

1.3. To avoid interference by unnecessary subject-experimenter contact, place only the screen, headphones/speakers and any input devices (keyboard, button boxes) inside the chamber. Have all interaction with the experimenter over intercom unless personal contact is required.

1.4. Use the following optimal parameters, based on extensive piloting, for background color and font size: grey background color (RGB: 125, 125, 125), black text color (RGB: 0; 0; 0), Arial font

face, size 27.

1.5. To reduce delays and jitter in visual presentation, use a video card and a monitor with a refresh rate of 100 Hz and higher.

1.6. To measure reaction times, use research-grade response pads, which have better ergonomics and more precise timing in comparison with conventional keyboards.

2. Stimulus preparation

2.1. Choose words of the language in question, which are controlled for their duration, lexical frequency and overall structure (to avoid any basic effects of surface stimulus properties on higher-level processing). Here, all base words were eight phonemes/letters long and consisted of three syllables with the CVCCVCVC structure (where C is consonant, and V is vowel).

2.2. To create multiple lists, divide the words into sets, which should not differ statistically (as measured with *t*-tests) on their lemma and syllabic frequency (these can be obtained from language-specific psycholinguistic databases)

NOTE: Here, Russian National Corpus http://www.ruscorpora.ru/en/ was used.

2.2.1. Here, use one set for creating (through modification) orthographically similar novel words and pseudowords, and use another set for creating unrelated control pseudowords, and another further set as unrelated control words (**Figure 2**). In such a design, it is recommended to have five sets of 10 items in each (50 stimuli in total).

2.3. To minimize any effects of surface forms on newly acquired semantics, counterbalance the sets across the subject pool, such that they play different experimental roles.

2.4. Create novel word forms such that they follow the rules of phonology and phonotactics and resemble existing words in terms of orthographic and phonological structure.

NOTE: To make sure that the new words can enter into competition with existing words, the current procedures were based on those developed in a series of experiments by Gaskell and colleagues[11,14] and aimed at keeping the word onsets (CVCCV-) stable, while rotating their offsets (-CVC) across different items in the set. That is, preserve the first two syllables of an existing word and vary the ultimate syllable such that a new, previously unfamiliar novel word form was created (e.g., mandarin –> mandanal*, where the last CVC was taken from another word in the list, cardinal, to create a new item).

2.5. Repeat the procedure described above for creating as many novel word forms as needed. Here, create a list of word forms to be learnt and a list of similar unlearnt pseudowords, (e.g. mandarin –> mandanal*, mandaket*, all three potentially entering into a lexical competition post-learning, as neighbors) as well as further control lists of words and novel pseudowords that

did not share this similarity (e.g., circular, muskenal*; Russian examples are used, transliterated from Cyrillic to Latin script for ease of understanding).

3. **Sentence stimuli for contextual semantic learning**

3.1. Create novel meanings to be associated with the new words in the process of learning. This could be made-up, obsolete or rare objects or concepts not present in the subjects' native language or culture.

3.2. For contextual learning of novel semantics, follow the procedures of Mestrez-Misse and colleagues[15] and create several sentences that describe situations through which one can understand the meaning of each of the novel words (e.g., "To control insects in medieval times, people used mandaket"). Use 5 unique sentences for each of the novel words, and gradually reveal the meaning of each new concept from a more general to more specific sentential context.

3.3. Present novel words ideally in their dictionary form (i.e., uninflected, e.g., singular nominative or accusative case in Russian), such that the surface form is not inflected differently in different sentences (**Table 1**), unless inflection rule learning is required.

3.3.1. Control and balance the length of the sentences and the number of words between conditions. Here, each sentence consisted of 8 words. Always place novel words at the end of the sentences. Such placement allows build-up of necessary contextual information (further, this allows implementing this design, if needed, in an EEG or MEG setting to record evoked brain responses unmasked by further word stimuli).

3.4. Present word-specific sentences in blocks of 5, gradually revealing the meaning of each or new words, without interleaving or randomizing sentences related to different novel words.

3.5. Randomize the order of the sub-blocks across the subject group. Word-by-word sentence presentation is recommended if the visual modality is used.

3.6. Determine the interstimulus interval based on specific stimulus properties to allow their convenient presentation; make sure to separate different sub-blocks with additional intervals and give regular breaks.

4. **Tasks to assess acquisition of new word forms and novel meanings**

NOTE: Use several tasks to assess different levels of acquisition and comprehension of both surface word forms and lexical semantics. Five tasks are used in the present protocol: free recall, cued recognition, lexical decision, semantic definition and semantic matching. The tasks are applied in the order they are listed below, which was optimized to reduce any carryover between successive tasks.

4.1. In the free recall task, have each participant reproduce as many new word forms as they

could remember by typing them into the prepared spreadsheet. The instruction is as follows: "Please write down in the column all the new words that you can remember."

4.2. Have the recognition and lexical decision (second and the third tasks, respectively) use the same stimuli and speed of presentation and differ only in instructions.

4.2.1. These tasks include all items (novel words, real competitor words the novel ones are derived from, untrained pseudoword competitors derived from the same real words, control unrelated pseudowords and control unrelated existing words).

4.2.2. For the recognition task, use the following instruction: "You will be presented with words sequentially. Press button "1" with the middle finger of the left hand if you have encountered the word during the experiment, or press "2" with the index finger of the left hand if you have not."

4.2.3. The instruction for the lexical decision task is: "You will be presented with real and meaningless words sequentially. Press "1" with the middle finger of the left hand if the word makes sense, or press "2" with the index finger of the left hand if it does not."

4.3. Use the semantic definition task to estimate the acquisition of novel meaning and the correspondence between the meaning and the surface form.

4.3.1. Give participants a list of the learnt items (i.e., those presented previously in the learning phase) with the instruction above: "Here is a list of new words presented to you previously. Try to define each of them and type their definitions into the spreadsheet".

4.3.2. To assess the completeness and accuracy of the given definitions, engage independent experts to rate the responses; agreement between experts could be tested using, e.g., Kendall's coefficient of concordance (W).

4.4. Use semantic matching task to assess the acquisition of semantics through making explicit links between the newly learnt word forms and their meanings in a simplified manner.

4.4.1. Use the following instruction: "You will be presented a word and three definitions. You should choose one correct definition for each word by pressing the corresponding button". Only one of the definitions is correct, with the other two corresponding to the other novel items. In addition to the three optional definitions, including "none of this" or/and "not sure" options are also recommended.

5. Procedures

5.1. Ensure that the tDCS stimulation precedes the behavioral task it is intended to modulate.

5.1.1. Wernicke's area.

NOTE: The electrode stimulation placement that corresponds to Wernicke's area is CP5 of the extended International 10-20 system for EEG[16,17].

5.1.1.1. To locate this location in the absence of an electrode cap, follow the standard 10-20 system procedures.

5.1.1.2. Measure the head with a tape from the inion to the nasion, and note the middle of this distance. Then, measure the distance from the left preauricular point to the right preauricular point, and mark the crosspoint of the two measurements.

5.1.1.3. To find the CP5 location, measure 30% of the distance between the preauricular points from the crosspoint down the left hemisphere and mark. Measure 10% of the distance between the inion and the nasion from the recently marked point to the back of the head. This point is the CP5 location for the active electrode (**Figure 3**).

5.1.2. Broca's area

NOTEL Closest to Broca's area is the F5 electrode site[18], or as the crossing point between T3-Fz and F7-Cz according to the 10-20 system.

5.1.2.1. In the absence of EEG cap, follow the standard 10-20 system procedures to find and mark the crosspoint between inion-nasion and preauricular points, described above.

5.1.2.2. To find the F5 location, measure 20% of the distance between the inion and the nasion from the crosspoint to the front of the head. Measure 30% of the distance between the preauricular points from the recently marked point down the left hemisphere. This point corresponds to the F5 location for the active electrode (**Figure 3**).

5.1.3. Homologous locations in the right hemisphere: for right-hemispheric homologues of Wernicke's and Broca's areas, use the same procedures as above, with the exception of measuring the distance from the midline down the right side of the scalp. Electrode locations are: CP6 for the RH Wernicke homologue and F6 for the Broca homologue.

5.1.4. Use spongy electrodes measuring 5 cm x 5 cm as this size is a good compromise between focal stimulation (which causes more irritation and discomfort) and larger electrodes that lack focality. Soak the electrodes in physiological saline solution for 5 min before application.

5.1.5. In order to minimize the effect of stimulation on other areas of the brain, place the reference electrode at the base of the neck on the left (right for homologues) side (see **Figure 3** and **Figure 4**). Use spongy electrodes measuring 5 cm x 5 cm as well.

NOTE: Particular attention should be paid to preventing the spreading of the solution beyond the boundaries of the electrode application zone. Special care should be taken to keep the

surrounding electrode area dry.

5.1.6. For optimal cathodal stimulation, use the following: 1.5 mA current for 15 min. At the onset, the current gradually rises from 0 to 1.5 mA over 30 s, and at the end of the stimulation it drops back to zero over 30 s.

5.1.7. For anodal stimulation, use the same procedure as cathodal stimulation, except the polarity is reversed, and the anodal electrode is placed at the active site, while the cathode is used as the reference electrode located outside the scalp area.

5.2. **Sham stimulation**

5.2.1. Perform the sham stimulation procedure generally as described above except that the current is only applied briefly in the beginning and the end of the sham session. To this end, during the first and the last 30 s of the session, apply an electric pulse of a triangular shape with a maximum of 1.5 mA in the present protocol.

5.3. Main behavioral task: contextual semantic learning

5.3.1. Present sets with contextual sentences for the novel words in a random order. Start each sentence with a word-by-word presentation.

5.3.2. After this, display the entire sentence on the screen to ensure its full understanding. Have participants press the spacebar with the index finger of the left hand after reading the whole sentence. Duration of sentence presentation is 5000 ms.

NOTE: Then a crosshair ("+") appears at the center of the screen for 500 ms and the next sentence begins. The sets of the sentences are separated from each other by appearance of three crosshairs ("+++") for 2000 ms. Each new concept presentation starts with another crosshair present for 500 ms before the sentence words are flashed. Each word is presented for 500 ms, the empty screen in the background color between words within one sentence for 300 ms.

5.4. Acquisition assessment procedure

5.4.1. To assess learning effects both immediately and following the overnight consolidation stage, break the stimulus set into two subsets, equally distributed across stimulus conditions and counterbalanced across the subject group, and run the assessment task immediately after the learning protocol on one subset, and after a 24 h delay on the other one.

NOTE: This strategy is based on the literature that highlights the importance of overnight memory consolidation for the acquisition of new words[19,20].

5.4.2. Use all developed tasks in the order described in section 3 above to assess different levels of word/concept acquisition. Choose the order of the tasks to minimize any carryover effects

from one task to the following ones.

5.4.3. For the tasks 1 and 4 use spreadsheets to be filled by subjects (by hand or using a text or spreadsheet processor); present the other tasks using temporally precise simulation software.

NOTE: Each stimulus in tasks 2 and 3 is presented for 600 ms, with a fixation cross ("+") present in the interstimulus interval (1400 ms); see **Figure 3**. For the other tasks the response time is not limited.

6. Data analysis

6.1. Perform data analysis using different tests comparing two sets of samples coming from continuous distributions (like Wilcoxon rank sum test or Mann-Whitney U-test) or medians (two-sample *t*-test, if the distribution is normal).

REPRESENTATIVE RESULTS:

While the data were analyzed for the specific set of tasks, it should be emphasized that the developed set of tests and the paradigm could be adapted to a variety of psycholinguistic experiments. The results were analyzed in terms of accuracy scores (number of correct answers) and the reaction time (RT) using non-parametric Wilcoxon signed-ranks test and Mann-Whitney U test across groups (cathodal and sham stimulation conditions). Significant differences for tasks within each group are presented in **Table 3**; below, we highlight the main stimulation-related results (for descriptive statistic see **Table 2**).

The comparison of performance in lexical decision task between the two groups (cathodal versus sham stimulation conditions) showed differences on the first day between accuracy for competitor pseudowords: accuracy increased more after cathodal than after sham stimulation ($p \leq 0.041$), suggesting reduced lexical competition after cathodal stimulation. In the recognition task, accuracy for novel words was better after sham than after cathodal stimulation both on the first ($p \leq 0.034$) and on the second ($p \leq 0.09$) day, suggesting reduced lexical learning efficiency after stimulation. Neither of the tasks showed differences in RT between groups. The results of the semantic tasks showed the matching between the novel form meaning and the surface form was more successful for cathodal group over sham on the second day only ($p \leq 0.011$).

Within each group, there were notable differences in accuracy scores and reaction times between the two assessment sessions. In the sham group, novel word recognition was better on the first than on the second day ($p \leq 0.049$). In the cathodal group, RT in the recognition task was significantly shorter for novel words than for competitor pseudowords on the first day ($p \leq 0.042$), but not on the second one. The results of lexical decision task showed that after cathodal stimulation on the first ($p \leq 0.003$) and on the second day ($p \leq 0.001$), there was better performance for novel words than for pseudoword competitors. In the sham group, however, this effect was observed on the second day only ($p \leq 0.002$).

FIGURE AND TABLE LEGENDS:

Figure 1: Experimental chamber.

Figure 2: Procedure for presenting stimuli in contextual learning sequence. (**a**) Making a stimuli groups: Groups of word/pseudoword stimuli. (**b**) Diagram of stimulus presentation in contextual learning block.

Figure 3: Localization of stimulation electrode for the Wernicke's and Broca's areas. Left panel: Side view and projection on brain areas. Brain zones, EEG electrodes (system 10–20%) corresponding to them, and red rectangles representing the the location of stimulating electrodes are marked. The reference electrode is shown at the base of the neck. Right panel: Projection of the stimulating electrode on the EEG system 10–20%

Figure 4: tDCS equipment. (**A**) Stimulator; (**B**) saline; (**C**) electrodes

Table 1: Examples of sentences for contextual learning of novel words.

Table 2: Descriptive statistics.

Table 3: Significant differences in accuracy scores and reaction times within each group (sham and cathodal stimulations). The values in parentheses are the mean scores and the reaction times.

DISCUSSION:

The results highlight a few important points that need to be taken into account when conducting psycholinguistic research in general, and neurolinguistics tDCS studies in particular. Stimulation of language cortices (exemplified here by Wernicke's area) produces a complex pattern of behavioral outcomes. Unlike the TMS technique, where it is possible to fully disrupt speech processing (e.g., the so-called "speech arrest" protocol)[21], this method enables a possibly more complex, graded and subtle influence on the language processing mechanisms. We have found a variety of both accuracy and reaction time differences which diverged substantially between conditions, tests and assessment days. The technical implications of the protocol reported are briefly discussed below.

To disengage the various effects, a battery of different tests is needed, which could test for processes at different levels of short- and long-term memory, lexical access, semantic processing, etc. For example, the effects here include different performance in accuracy of recall and recognition for different stimulus types and stimulation conditions, which suggests differential lexical competition effects for novel and old items, and diverging effects of tDCS at lexical and semantic levels. Our results confirm sensitivity of the utilized tasks to efficiency of novel word acquisition at different levels, including recognition, understanding of a word meaning and free recall.

In the same way, a tDCS condition (e.g., anodal, cathodal stimulation) requires a proper control condition (or control group), sham (placebo) stimulation being the most appropriate baseline. Unlike electrical stimulation of the motor cortex, the effects may not always be unambiguous[22], they strongly depend on the tests used, and the effects may not appear at all[23].

Another very important point is that only one type of stimulation could be applied in each individual subject in the context of a single experimental session. This normally entails a between-group design, for instance, an anodal stimulation group, a cathodal stimulation group, and a placebo (called sham) control group. For within-group designs, use different tDCS protocol on different days, at least 24 h apart (in learning studies, this also entails using different linguistic stimuli on different days to avoid contamination of results by repetition effects). The present report uses an experiment with cathodal stimulation of Wernicke's areas as an example, but similar procedures apply to other polarities/sites.

Contextual presentation of new words significantly expands the possibilities of simultaneous study of acquisition of word form per se and of its semantics. Traditionally, these processes are studied separately focusing either on the acquisition of a new word form or on the correlation of a meaning of a familiar word with other semantic units[24–26]. The proposed protocol combines both aims; therefore, it is possible to compare the dynamics of a new concept acquisition at the level of word form perception and that of mastering its content, which is achieved by using a comprehensive set of tests. The need for such a comparison is emphasized here by diverging dynamics of performance on novel surface forms recall and recognition as opposed to semantic matching.

It is important to remember the main differences between tDCS and other non-invasive brain stimulation methods, such as TMS. Since there is no simple way to determine individual sensitivity to tDCS by threshold assessment a single protocol is applied for all subjects. It is very difficult to accurately estimate the stimulation area—one can only speak about the hypothetical area. It is also difficult to estimate the duration of offline stimulation effects after the current is turned off. Presumably, the main effects of stimulation are observed up to one hour after the termination of stimulation. However, the effects of stimulation can sometimes be detected even one day after the stimulation[20].

Yet, compared with TMS, the relative ease of application of tDCS, the significantly lower probability of muscle-related side effects and the absence of acoustic artifacts make this protocol attractive for studying speech functions. It is also worth noting that the combination of electrical stimulation with other methods, for example with TMS, fMRI and pharmacological intervention, allows studying neuronal mechanisms of tDCS in more detail[27,28].

Since tDCS stimulation is not highly localized, a non-specific effect is possible. This is indicated by the analysis of literature data, when very different or even opposite protocols may sometimes lead to similar results. This may be due to the general impact on other cognitive functions and processes such as attention, retrieval from memory, and so on. A specialized battery of tests is needed to detect the effects associated with a particular language feature. Following the

proposed steps of the stimulus material creation (verification of the surface or lemma frequency of the words, length of words and sentences, etc.) it is necessary to consider the grammatical and phonetic structure of a language. For instance, the number of words in a sentence and the length of the words can vary depending on the exact need. In addition, the words used in the experiment should be controlled for both spelling and sound. In an orthographically transparent language such as Russian, this is relatively straightforward, but it may be difficult to attain in other languages (e.g., English, Danish or Mandarin).

In line with a body of previous studies, we find different effects of acquisition immediately after the learning block and after an overnight sleep, which highlights effects of overnight consolidation. Importantly, we also find group differences (sham versus cathode) on the second day. It is generally accepted that the physical effect of stimulation of the cortex is relatively short-lasting, on the order of minutes to several hours. This implies that the cognitive effects achieved during the transient stimulation phase are nevertheless maintained over a longer period and may therefore be possibly used for modulating word acquisition and processing in practical settings. Obviously, not only the core language areas of Broca and Wernicke are involved in the language function; adoption of the protocol described above is possible for any area of the brain, while a battery of psycholinguistic tests fine-tuned for specific experimental purposes is still needed to assess the stimulation impact on a specific neurolinguistic trait.

ACKNOWLEDGMENTS:
Supported by RF Government grant contract No.14.W03.31.0010.

DISCLOSURES:
The authors have nothing to disclose.

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Figure 1

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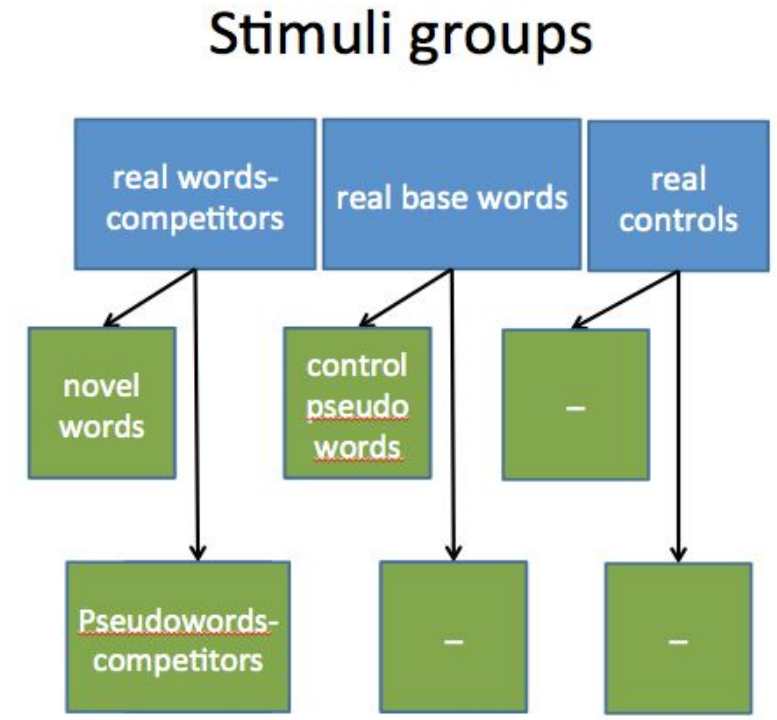
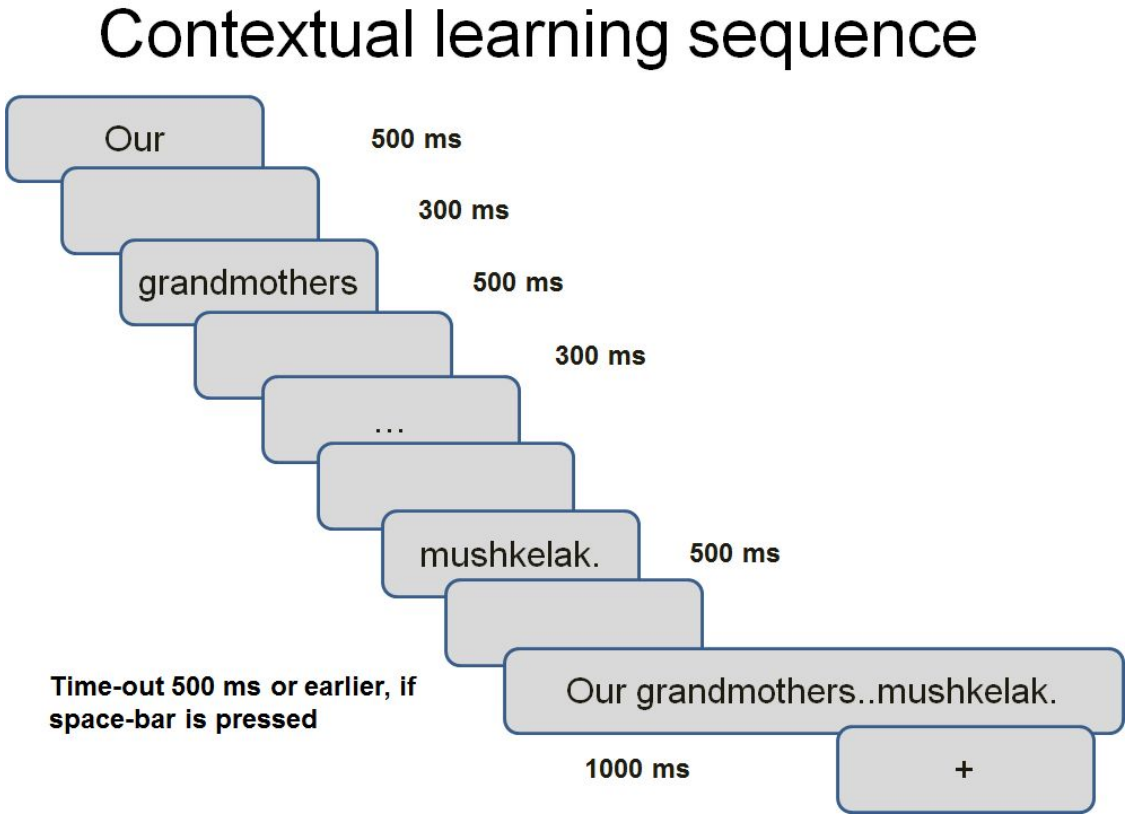


a. Groups of word/pseudoword stimuli



b. Diagram of stimulus presentation in contextual learning block

Figure 3

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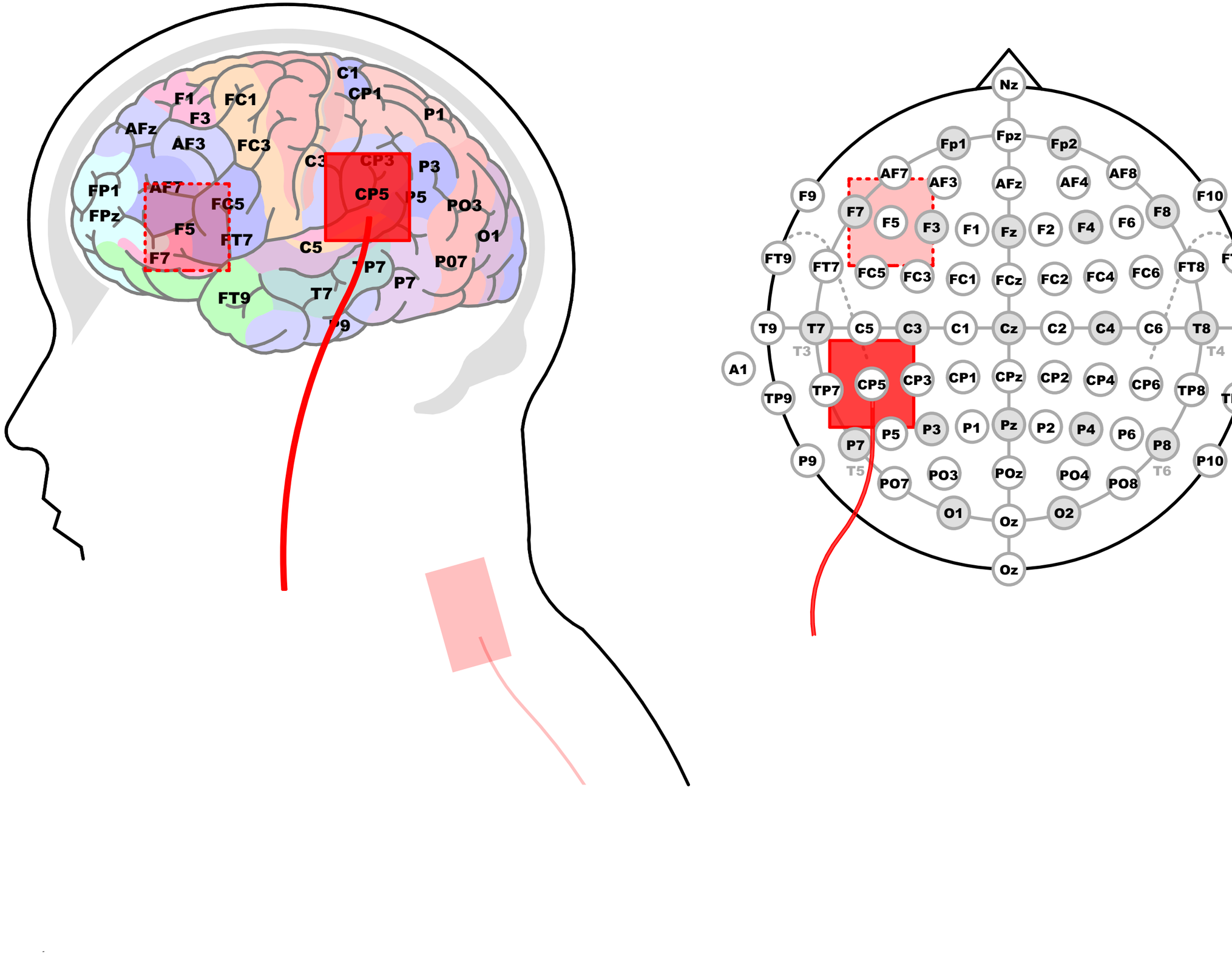

Figure 4

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Table 1 Click here to access/download;Table of Materials;Table 1.xlsx

Examples of sentences
Нашим бабушкам было неведомо такое чувство как мушкелак. Our grandmothers did not know such as feeling as mushkelak.
Благодаря своей хорошей памяти. Маша не чувствовала мушкелак. Thanks to her good memory. Masha never experienced any mushkelak.
Заведя сразу несколько аккаунтов. я начал испытывать мушкелак. Having got a few accounts. I started suffering from mushkelak.
Секретный блокнот поможет решить такую проблему как мушкелак. A secret notebook could help you solve the problem of mushkelak.
Петр устанавливал одинаковые пароли. не желая ощущать мушкелак. Peter always set the same password as he did not want to have any mushkelak.

Table 2 Click here to access/download;Table of Materials;Table 2.xlsx

	Sham stimulation	p-value
Task 1: free recall. Acc		
Between days	Accuracy scores Day 1 (4.91) vs. accuracy scores Day 2 (2.53)	0.001
Task 2: recognition. Acc		
Day 1	Novel words (3.06) vs.	–
	Competitor words (3.63)	0.042
	Control pseudowords (3.79)	0.041
	Control words (4.67)	0.001
	–	–
Day 2	Novel words (2.58) vs.	–
	Competitor words (4.40)	0.001
	Control pseudowords (4.33)	0.001
	Control words (4.58)	0.001
	–	–
Between days	Novel words (3.06 vs. 2.58)	0.049
	Competitor words (3.63 vs.4.40)	0.011
	Competitor pseudowords (2.60 vs. 3.13)	0.034
	Control pseudowords (3.79 vs. 4.33)	0.030
Recognition. Reactio		
Day 1	Novel words (793) vs. Control words (699)	0.005
Day 2	Novel words (837) vs. Control words (734)	0.007
Task 3: lexical decision. A		
Day 1	Novel words (2.417) vs.	–
	Competitor words (4.125)	0.001
	Control pseudowords (4.21)	0.001
	Control words (4.54)	0.001
	–	–
Day 2	Novel words (2.042) vs.	–
	Competitor words (4.375)	0.001
	Competitor pseudowords (3.813)	0.002

	Control pseudowords (4.54)	0.001
	Control words (4.42)	0.001
Between days	No significant differences	–
Lexical decision. React		
Day 1	Novel words (817) vs.	
	Competitor words (747)	0.022
	Competitor pseudowords (927)	<0.001
	Control pseudowords (891)	0.033
Day 2	Novel words (878) vs.	–
	Competitor words (743)	0.003
	Control words (719)	0.008
Task 4: semantic definition. Match		
Between days	Matching scores Day 1 (1.27) vs. matching scores Day 2 (0.52)	0.001
	Accuracy scores Day 1 (7.97) vs. accuracy scores Day 2 (2.82)	0.001
Task 5: semantic matching.		
Between days	Accuracy scores Day 1 (3.16) vs. accuracy scores Day 2 (2.41)	0.006
Semantic matching. Rea		
Between days	React Reaction time on Day 1 (10914) vs. reaction time Day 2 (8798)	0.002

Cathodal stimulation	p-value
curacy scores	
Accuracy scores Day 1 (5.69) vs. accuracy scores Day 2 (2.84)	0.000
curacy scores	
Novel words (1.96) vs.	–
Competitor words (3.73)	0.004
Competitor pseudowords (2.69)	0.045
Control pseudowords (3.92)	0.002
Control words (4.29)	0.000
Novel words (1.56) vs.	–
Competitor words (4.10)	0.000
Competitor pseudowords (3.31)	0.001
Control pseudowords (4.50)	0.000
Control words (4.38)	0.000
Competitor words (3.73 vs. 4.10)	0.036
Competitor pseudowords (2.69 vs. 3.31)	0.024
Control pseudowords (3.92 vs. 4.50)	0.020
–	–
n time (ms)	
Novel words (858) vs.	–
Competitor pseudowords (962)	0.042
Control words (767)	0.006
Novel words (933) vs.	–
Competitor words (818)	0.001
Control pseudowords (866)	0.045
Control words (817)	0.014
Accuracy scores	
Novel words (1.958) vs.	–
Competitor words (4.104)	0.000
Competitor pseudowords (4.021)	0.003
Control pseudowords (4.25)	0.001
Control words (4.54)	0.000
Novel words (1.563) vs.	–
Competitor words (4.458)	0.000
Competitor pseudowords (3.93)	0.001

Control pseudowords (4.58)	0.000
Control words (4.63)	0.000
Control pseudowords (4.25 vs. 4.58)	0.033
ion time (ms)	
Novel words (921) vs.	–
Competitor words (796)	0.001
Control words (784)	0.013
–	–
Novel words (962) vs.	–
Competitor words (811)	0.003
Control words (756)	0.001
ing and Accuracy scores	
Matching scores Day 1 (1.87) vs. matching scores Day 2 (1.39)	0.006
Accuracy scores Day 1 (8.71) vs. accuracy scores Day 2 (5.86)	0.000
. Accuracy scores	
No significant differences	–
ction time (ms)	
Reaction time Day 1 (10856) vs. reaction time Day 2 (8908)	0.015

Table 3 Click here to access/download;Table of Materials;Table 3.xlsx

	Sham stimulation	p-value
Task 1: free recall. Acc		
Between days	Accuracy scores Day 1 vs. accuracy scores Day 2	0.001
Task 2: recognition. Ac		
Day 1	Novel words vs.	–
	Competitor words	0.042
	Control pseudowords	0.041
	Control words	0.001
	–	–
Day 2	Novel words vs.	–
	Competitor words	0.001
	Control pseudowords	0.001
	Control words	0.001
	–	–
Between days	Novel words	0.049
	Competitor words	0.011
	Competitor pseudowords	0.034
	Control pseudowords	0.030
Recognition. Reactio		
Day 1	Novel words vs. Control words	0.005
Day 2	Novel words vs. Control words	0.007
Task 3: lexical decision. A		
Day 1	Novel words vs.	–
	Competitor words	0.001
	Control pseudowords	0.001
	Control words	0.001
	–	–
Day 2	Novel words vs.	–
	Competitor words	0.001
	Competitor pseudowords	0.002
	Control pseudowords	0.001
	Control words	0.001

Between days	No significant differences	–
Lexical decision. React		
Day 1	Novel words vs.	
	Competitor words	0.022
	Competitor pseudowords	<0.001
	Control pseudowords	0.033
Day 2	Novel words vs.	–
	Competitor words	0.003
	Control words	0.008
Task 4: semantic definition. Match		
Between days	Matching scores Day 1 vs. matching scores Day 2	0.001
	Accuracy scores Day 1 vs. accuracy scores Day 2	0.001
Task 5: semantic matching.		
Between days	Accuracy scores Day 1 vs. accuracy scores Day 2	0.006
Semantic matching. Rea		
Between days	Reaction time Day 1 vs. reaction time Day 2	0.002

Cathodal stimulation	p-value
curacy scores	
Accuracy scores Day 1 vs. accuracy scores Day 2	<0.001
curacy scores	
Novel words vs.	–
Competitor words	0.004
Competitor pseudowords	0.045
Control pseudowords	0.002
Control words	<0.001
Novel words vs.	–
Competitor words	<0.001
Competitor pseudowords	0.001
Control pseudowords	<0.001
Control words	<0.001
Competitor words	0.036
Competitor pseudowords	0.024
Control pseudowords	0.020
–	–
n time (ms)	
Novel words vs.	–
Competitor pseudowords	0.042
Control words	0.006
Novel words vs.	–
Competitor words	0.001
Control pseudowords	0.045
Control words	0.014
Accuracy scores	
Novel words vs.	–
Competitor words	<0.001
Competitor pseudowords	0.003
Control pseudowords	0.001
Control words	<0.001
Novel words vs.	–
Competitor words	<0.001
Competitor pseudowords	0.001
Control pseudowords	<0.001
Control words	<0.001

Control pseudowords	0.033
ion time (ms)	
Novel words vs.	–
Competitor words	0.001
Control words	0.013
–	–
Novel words vs.	–
Competitor words	0.003
Control words	0.001
ing and Accuracy scores	
Matching scores Day 1 vs. matching scores Day 2	0.006
Accuracy scores Day 1 vs. accuracy scores Day 2	<0.001
. Accuracy scores	
No significant differences	–
ction time (ms)	
Reaction time Day 1 vs. reaction time Day 2	0.015

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3. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We have tried to do it as much as possible.

4. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

We have tried to reformat it as much as possible. However, other experimental languages will most likely require some protocol changes. That is why mandatory steps in brain stimulation are described unambiguously, but some behavioral tests require adaptation to the subject's language.

5. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.

Thanks for the comment. We have now significantly changed the protocol following the suggestions above.

6. 4.1: What are the inclusion and exclusion criteria of the participants?

Thank you for your comment. We have added additional paragraph to the text of the paper to describe inclusion and exclusion criteria regarding health, native language and handedness of experimental participants: "1.1 Subjects: in a typical language experiment, all subjects

must be right-handed and have no record of language deficits, neurological or psychiatric disorders. Their native language must be controlled.".

7. Figure 2: Please describe panels a and b in the figure legend.

Thank you for pointing out the omission to us, this has now been fixed

8. Figure 3: Please describe what the left and right panels represent in the figure legend. Also please explain what the different colors and the red box represent.

Thank you, this has been done.

9. Figure 4: Please label the different parts in the figure.

The labels have now been introduced, as requested.

10. JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

Thanks a lot for the pointing that out. We have completely revised the discussion part according to your suggestions and have now focused in on the protocol using precisely the points listed above.

11. References: If there are six or more authors, list the first author and then "et al.".

We have re-formatted the references style according to your instructions. We are using Mendeley software with the style "Journal of Visualized Experiments"

12. Table of Materials: Please include the name, company, and catalog number of all relevant supplies, reagents, equipment and software in separate columns in an xls/xlsx file. Please sort the items in alphabetical order according to the name of material/equipment.

We have attached this file.

Changes to be made by the Author(s) regarding the video:

1. Please update the video based on the revised protocol. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

2. Please note that the protocol portion of the current video is composed of mostly interviews and descriptions. Please minimize the interview sessions from the protocol and instead focus the protocol section on showing how to perform the specific actions.

• 3:19 - Instead of "EXEMPLES", it should be "EXAMPLES".

• 9:18, 9:44 - The edits here are jump cuts, which tend to have a jarring effect on the viewer. They should be smoothed out with crossfades instead.

• 12:49 - A chapter title card that reads "Conclusions" should be added here.

• The article ID number (59159) should be added to the file name for any future submissions.

3. Please upload a revised high-resolution video here:
https://www.dropbox.com/request/VBTGYCeFWh2LMEg2GtZw

Thank you very much, we have corrected the video as instructed.

Reviewer #1:
This is an excellent and well-written proposal for the experimental modulation of Broca's and Wernicke's brain areas in humans using non-invasive transcranial direct current stimulation. The study is also very well illustrated.
-A possible difficulty of this proposal is the proper location of Broca's and Wernicke's brain areas and the different effects of selected DCs depending on minor differences in cortical surfaces and sulcus. A comment on this point should be included in the text.

Thanks for the positive assessment of our paper and for the very useful comment above. We have included a discussion of this issue in the revised the text of the paper. Indeed, the location of the electrode over the indicated brain sites does not mean direct stimulation of only this area - whereas the pattern of electrical distribution is rather complicated and is beyond the frame of this paper, most tDCS studies assumed that the maximum effect from stimulation is expected under the electrode. This is an oversimplification that should be taken with caution; in addition, one should keep in mind individual differences between cortical surfaces of different subjects which further complicates neuroanatomical precision of this method.

-While the size of the active electrodes seems to be a little be large, the side of the indifferent electrode is proportionally rather small. Please check these sizes with reports using similar approaches in humans.

Thank you for your comment. A larger stimulation area means less current per unit of surface. On the other hand, by reducing the size of the electrode, we increase the pain effect of stimulation. Based on our own and literature data, we estimate that an electrode size of 5 to 5 cm is an optimal compromise between the focality of the stimulation and the subject's comfort. In addition (cf. the previous comment), considering the lack of spatial precision of tDCS and individual variability, larger electrodes help ensure that the current is delivered to the target area. These considerations are now specified in the revised manuscript.

-A video should be provided describing the main procedures and/or illustrating the main details of this interesting experimental approach. Sorry, if the video is already provided, because I could not find it!

When submitting the article we uploaded the full version of the video version and are very sorry to hear that you have not been able to locate it. We will alert the editor to this issue.

-Authors described some interesting effects evoked by cathodic stimulations. In a recent study carried out in behaving rabbits has been reported similar effects when applying TDC cathodic currents on the somatosensory cortex. These effects seemed to be mediated by the activation of adenosine A1 receptors (Marquez-Ruiz et al., PNAS USA, 2012)

Thank you very much for alerting us to this interesting corroborating evidence. We have now added this citation to the revised version of the paper. Unfortunately, according to the rules of this journal (protocol-focussed reports rather than research papers), we cannot discuss the results of stimulation in detail. Therefore, we now briefly refer to the possibility of determining the possible mechanisms causing the effects of electrical stimulation:

"It is also worth noting that the combination of electrical stimulation with other methods, for example with TMS, fMRI and pharmacological intervention, allow to study the neuronal mechanisms of tDCS (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Márquez-Ruiz et al., 2012)."

Reviewer #2:

Summary: The authors describe the use of transcranial direct current stimulation (tDCS) over Wernicke's area after a word acquisition experiment to modulate performance in five different behavioural tasks aimed at measuring learning success. Both psycholinguistic and tDCS-related aspects relevant for such a study are discussed. This is a concise manuscript which highlights many important considerations for conducting combined tDCS and psycholinguistic studies. However, I have a couple of questions and points that require clarification.

Thank you very much for this positive assessment of our paper and for your constructive remarks, which we address below.

Major concerns:
1. In the introduction, it is stated that the location of language-related areas does not lend itself to TMS. I think this claim is not warranted or in any case needs to be explained more clearly, because in fact TMS is more reliable in focally targeting specific cortical regions whereas tDCS causes very diffuse and unpredictable neurophysiological and behavioural effects. So while there obviously are procedural advantages of tDCS, focality definitely is not one of them.

Thank you for this comment. We apologize if our formulation was misleading, and have changed it now. By no means can we suggest that tDCS is more focal than TMS. The issue with TMS of language areas is that (unlike e.g. TMS of hand or foot motor cortex) it can cause pain to the subject , due to the close proximity of the muscles to the point of stimulation. Furthermore, during online TMS protocols, sound artifacts from stimulation appear, which may interfere with the linguistic stimulation. We have now changed our argumentation in the revised manuscript to make it clear - many thanks again.

We have added:

"It is important to remember the main differences between tDCS and other non-invasive brain stimulation methods, such as TMS.

1. Since there is no simple way to determine individual sensitivity to tDCS by threshold assessment, a single protocol is applied for all subjects.

2. It is very difficult to accurately estimate the stimulation area - one can only speak about the hypothetical area.

3. It is also difficult to estimate the duration of offline stimulation effects. Presumably, the main effects of stimulation are observed up to one hour after the termination of stimulation. However, the effects of stimulation can sometimes be detected even one day after the stimulation [20].

Yet, compared with TMS, the relative ease of application of tDCS, the significantly lower probability of pain effects and the absence of acoustic artifacts make this protocol attractive for studying speech functions.

It is also worth noting that the combination of electrical stimulation with other methods, for example with TMS, fMRI and pharmacological intervention, allows studying the neuronal mechanisms of tDCS in more detail [27,28]."

2. Throughout the manuscript, the authors refer to optimal parameters for different procedures (e.g. font size for visual stimuli, stimulation intensity and length). However, it is not clear to me where these "optimal" values come from so I think it would be useful to elaborate a bit more on that - was it from pilot studies, other fundamental work etc.?

Thank you. Indeed, the parameters come from the previous work as well as our own extensive piloting. Note, however, that as per JoVE policy, the main focus of the paper (including video) is on the detailed description of experimental protocols within minimum space limits, whereas the protocol development is per se outside the journal's focus. As stated in the official requirements of the journal, 'JoVE publishes novel methods, innovative application of existing techniques, and gold standard protocols that enable a greater level of experimental transparency. A detailed text protocol and representative results accompany every video to further expand the impact of our video articles.' Therefore, we omitted the details of the development of the experimental protocol, since this is not within the scope of this paper. However, we did a large number of pilot experiments, where we checked various stimulation parameters and asked the subjects to evaluate their comfort during the experiment. Based on this, we have given in the paper the parameters of our protocol. The tDCS protocol was taken on the basis of our experience in stimulating the motor cortex — when it is applied, there are significant changes in the thresholds of motor responses. In addition, also from the literature data, this protocol is successfully used for psycholinguistic studies.

3. I'm not entirely sure what the structure for these types of articles should be, but upon reading the manuscript, I was expecting some sort of hypothesis section. It is only described what is being done and what the results were, but no indication was given as to what kind of effects were expected. In the easiest case, this would entail a predicted performance decrement following cathodal tDCS (although research has shown that the anodal = improvement/cathodal = decrease is not as straight-forward for higher cognitive functions, see e.g. Jacobson, Koslowsky & Lavidor, Exp Brain Res 2012), but maybe the authors also have more specific hypotheses with respect to the different tasks.

Thank you. As mentioned above, JoVE video articles are focussed on protocols, not on theoretical aspects of research. (For instance, the instruction for authors says that "JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Focus on: a) Critical steps within the protocol b) Any modifications and troubleshooting of the technique and ...") For this reason, we have now had, on Editor's request,

to remove even the minimal discussion of psycholinguistic implications from the revised manuscript. Therefore, in the results and discussion, we are only allowed to give an example of how our protocol can be tested, not a theory-based overview of the research field and findings. Therefore, not all of the findings can be presented in this article. The purpose of this paper was to demonstrate the use of tDCS protocols to stimulate speech zones. We have now significantly changed the "Discussion" section in line with this ,following the requirement by the editorial team. We have now further enhanced the discussion of these issues in the revised manuscript and included the relevant literature references:

“The same way, a tDCS condition (e.g. anodal, cathodal stimulation) requires a proper control condition (or group), sham (placebo) stimulation being the most appropriate baseline. Unlike electrical stimulation of the motor cortex, the effects may not always be unambiguous (Jacobson, Koslowsky, & Lavidor, 2012), they strongly depend on the tests used, and the effects may not appear at all (Malyutina et al., 2018)”.

4. The authors say that tDCS studies are typically conducted in between-group designs (top paragraph of page 5). This is not necessarily the case, as many studies also employ within-group designs in which participants receive different forms of tDCS in different sessions. It may be worthwhile to briefly discuss the advantages and disadvantages of both methods.

Thank you very much for raising this important point. Indeed, tDCS experiments can be conducted as both within-group (e.g. with different zones on different days) and between-group (different zones in different subjects) design. In situations when the same stimulation protocol cannot be used twice, as in the learning paradigm here, a between-group design is suggested. We have now made this matter fully clear in the revised manuscript.

5. The procedural details for the tDCS application need some clarification.
5a. It is not clear to me why the locations are measured manually when they eventually correspond to 10-20 positions anyway. I think it is easier - and also common practice - to say that the electrode is placed under the respective electrode position of an EEG cap. The method isn't focal enough to distinguish between slight variations in placement anyway, it should only be emphasised that the electrode should not be moved throughout the experiment/stimulation.

Thank you. We have now clarified this in the revised text. One of the goals of the paper (including the video protocol) is to show how to conduct this stimulation. Possible readers may not only be experts in the field of tCS and EEG, but scientists who try this technique for the first time. In addition, it is possible that not all laboratories are simultaneously equipped with TES and EEG caps. Therefore, we felt it could be useful to explain how the locations are established using the conventions of the 10-20 system.

5b. I first thought that the study would compare tDCS across Broca's and Wernicke's area and their RH homologues, as these locations are mentioned in sections 5.1.1-3. It may be better to emphasise at this point that this study investigates the effects of tDCS over Wernicke's area, but of course other target sites are also possible and may be operationalised in the following manner.

Of course, there are a number of areas of the brain associated with speech. We chose the classical language area of Wernicke to demonstrate the effects of stimulation. As mentioned

above, the main focus of such short video-papers is on protocol. We therefore describe the tDCS of both Broca's and Wernicke's areas (as well as their RH homologues) and use Wernicke's area to demonstrate an example result of this protocol. We have now tried to make this clear in the revised paper.

5c. Point 5.1.4. mentions that 5x5 cm electrodes are used, whereas point 5.1.5. mentions 5x2 cm.

Thank you very much pointing out this inconsistency - it has now been corrected in the revised paper.

5d. Instructions as to the placement of the "reference" electrode should be kept consistent: If for LH-tDCS it is placed on the left side of the neck, I think it should be placed on the right side of the neck for RH-tDCS.

Thank you very much, we have corrected the manuscript according to this comment

Minor concerns:
1. Keyword should read tDCS instead of tDSC.
2. P.2, line 104: "seminal" instead of "seminar"
3. P.2, line 106 and others: Not sure what authors mean by "camera" - maybe "chamber"?
4. P.5, line 239 and elsewhere: µA should read mA!
5. Data analysis: The first paragraph sounds as if these tests are the only possible analysis methods, which is not the case. Also, if I understand correctly, the study presents a 2x2 design (tDCS [cathodal vs. sham] x time point [immediately after stimulation vs. next day]), so this is something that should be made more explicit. Also, there seems to be a word missing before "medians" in line 279.
*6. Table 2: I think it would be useful to split this in two tables (or at least rearranging the contents), with one reporting the descriptives (*including* a measure of variance!) and the other reporting the results from the inferential statistics. The way the data are presented now is very unintuitive.*

Thank you very much for these useful corrections and suggestions. We have now implemented all of these comments in the revision.

Once again, we wish to thank the Reviewers and Editoral&Production team sincerely for their work, positive comments and constructive criticisms that were of uttermost help in improving the paper. In sum, we believe that we have been able to meet all concerns expressed by these valued colleagues in response to our original submission. Attached to this letter is the revised version of the manuscript, which we hope will be found acceptable for publication.