Journal of Visualized Experiments

Stimulation Location Determination using a 3D Digitizer with High-Definition Transcranial Direct Current Stimulation

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE60263R2
Full Title:	Stimulation Location Determination using a 3D Digitizer with High-Definition Transcranial Direct Current Stimulation
Section/Category:	JoVE Neuroscience
Keywords:	neuroscience; Noninvasive Brain Stimulation; HD-tDCS; 3D digitizer; the spatial registration; rTPJ
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Chongqing, China

TITLE:

Stimulation Location Determination using a 3D Digitizer with High-Definition Transcranial Direct Current Stimulation

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KEYWORDS:

neuroscience, noninvasive brain stimulation, HD-tDCS, 3D digitizer, spatial registration, rTPJ

SUMMARY:

Presented here is a protocol to achieve higher accuracy in determination of stimulation location combining a 3D digitizer with high-definition transcranial direct current stimulation.

ABSTRACT:

The abundance of neuroimaging data and rapid development of machine learning has made it possible to investigate brain activation patterns. However, causal evidence of brain area activation leading to a behavior is often left missing. Transcranial direct current stimulation (tDCS), which can temporarily alter brain cortical excitability and activity, is a noninvasive neurophysiological tool used to study causal relationships in the human brain. High-definition transcranial direct current stimulation (HD-tDCS) is a noninvasive brain stimulation (NIBS) technique that produces a more focal current compared to conventional tDCS. Traditionally, the stimulation location has been roughly determined through the 10-20 EEG system, because determining precise stimulation points can be difficult. This protocol uses a 3D digitizer with HD-tDCS to increase accuracy in determination of stimulation points. The method is demonstrated using a 3D digitizer for more accurate localization of stimulation points in the right temporo-parietal junction (rTPJ).

INTRODUCTION:

Transcranial direct current stimulation (tDCS) is a noninvasive technique that modulates cortical excitability with weak direct currents over the scalp. It aims to establish causality between neural excitability and behavior in healthy humans[1-3]. In addition, as a motor neurorehabilitation tool, tDCS is widely used in the treatment of Parkinson's disease, stroke, and cerebral palsy[4]. Existing evidence suggests that traditional pad-based tDCS produces current flow through a relatively larger brain region[5-7]. High-definition transcranial direct current stimulation (HD-tDCS), with the center ring electrode sitting over a target cortical region surrounded by four return electrodes[8,9], increases focality by circumscribing four ring areas[5,10]. In addition, changes in excitability of the brain induced by HD-tDCS have significantly larger magnitudes and longer durations than those generated by traditional tDCS[7,11]. Therefore, HD-tDCS is widely used in research[7,11].

Noninvasive brain stimulation (NIBS) requires specialized methods to ensure that a stimulation site is present in the standard MNI and Talairach systems[12]. Neuronavigation is a technique that allows for mapping interactions between transcranial stimuli and the human brain. Its visualization and 3D image data are used for precise stimulation. In both tDCS and HD-tDCS, a common assessment of stimulation sites on the scalp is typically the EEG 10-20 system[13,14]. This measurement is widely used for placing the tDCS pads and optode holders for functional near infrared spectroscopy (fNIRS) in the initial stage[13-15].

Determining the precise stimulation points when using the 10-20 system can be difficult (e.g., in the temporo-parietal junction [TPJ]). The best way to solve this is to obtain structural images from participants using magnetic resonance imaging (MRI), then obtain the exact probe position by matching target points to their structural images using digitizing products[15]. MRI provides good spatial resolution but is expensive to use[15-17]. Moreover, some participants (e.g., those with metal implants, claustrophobic people, pregnant women, etc.) cannot be subjected to MRI scanners. Therefore, there is a strong need for a convenient and efficient way to overcome the abovementioned limitations and increase accuracy in determining stimulation points.

This protocol uses a 3D digitizer to overcome these limitations. Compared to MRI, key advantages of a 3D digitizer are low costs, simple application, and portability. It combines five reference points (i.e., Cz, Fpz, Oz, left preauricular point, and right preauricular point) of individuals with location information of the target stimulation points. Then, it produces a 3D position of electrodes on the subject's head and estimates their cortical positions by fitting with the vast data from the structural image[12,15]. This probabilistic registration method enables the presentation of transcranial mapping data in the MNI coordinate system without recording a subject's magnetic resonance images. The approach generates anatomical automatic labels and Brodmann areas[11].

The 3D digitizer, used to mark space coordinates based on the data from structural images, was first used to determine the position of optodes in fNIRS research[18]. For those who use HD-tDCS, a 3D digitizer breaks the finite stimulation points of the EEG 10-20 system. The distance of the four return electrodes and center electrode is flexible and can be adjusted as needed. When using the 3D digitizer with this protocol, the coordinates of the rTPJ were obtained, which is beyond the 10-20 system. Also shown are the procedures for targeting and stimulating the right temporo-parietal junction (rTPJ) of the human brain.

PROTOCOL:

The protocol meets the guidelines of the Institutional Review Board of Southwest University.

1. Determination of stimulation location

1.1. Review the literature and confirm the stimulation location (here, the rTPJ)[19-21].

2. Preparation of electrode holding cap

NOTE: The following steps are shown in **Figure 1**.

2.1. Ensure that all necessary materials are readily available: the 3D digitizer (**Figure 2**), standard measurement tape, a marking pen, the headform, and a swimming cap.

2.2. Put the cap on the headform and mark the points on the cap.

2.2.1. Localize the Vertex (Cz). To do so, first mark the midpoint of the distance between the nasion and inion using a skin marker[13,14,22]. Then, measure the distance between the pre-auricular points and mark the midpoint. The point at which both points intersect is the Cz.

2.2.2. Check the location of the center electrode and the return electrodes. Here, the stimulation was applied on rTPJ. The rTPJ roughly corresponds to the midpoint between CP6 and P6 in the 10-10 EEG system[19-21].

2.2.3. Find CP6 and P6[22-25]. According to the proportional requirements of the 10-10 system, locate the approximate location of the rTPJ on the scalp and mark it on the cap.

2.2.4. Adjust the radius of the four return electrodes based on the objectives[11,14,26]. After this decision, mark the center electrode and return electrode locations on the cap.

3. 3D digitizer measurement

3.1 Scan with the metal scanner to ensure that the environment for 3D digitizer is metal-free.

3.2 Placement of the cap on the subject's head

3.2.1. Make sure that the references (Cz, Fpz, Oz, left preauricular point, and right preauricular point) on the cap align with the international 10-10 system for scalp location[22]. For example, localize the Vertex (Cz) on the scalp and place the cap onto the subject's head, aligning the cap's Cz to the subjects.

3.3 Arranging the 3D digitizer equipment

3.3.1 Connect the 3D digitizer to the computer using the Universal Serial Bus (USB) interface and make sure that the digitizer software is available and ready[27].

3.3.2 Put the source in front of the subject and fasten the elastic rope of the sensor around the head. Importantly, make sure that neither the source nor sensor moves during 3D digitizer measurement.

NOTE: The source is a magnetic transmitter that emits an electromagnetic dipole field. The sensor is a receiver that detects the field.

3.3.3 Open the digitizer software on the computer and make sure that the 3D digitizer system communicates with the software.

3.3.4 Test the accuracy of the stylus. Find a length of 10 m on the ruler and record the zero graduation and ten graduation, respectively, using the stylus.

NOTE: The measurement distance between the two recording points of the 3D digitizer should be captured. Compare the error with the reading from the 3D tracker.

3.3.5 Select the **New** icon and create a new subject file. Select the **Sessions** box, then **Reference**.

NOTE: Using the 3D digitizer stylus, the reference position data (Cz, inion, nasion, left ear, right ear) of the subject are collected according to the software prompts.

3.3.6 To cater to the requirement of fNIRS experiments, use the **Transmitter**, **Detector,** and **Channel** options. Collect the position data of the center electrode and four return electrodes 3x for the transmitter, detector, and channel, in order to reduce error. Ensure that five electrodes are numbered and localize in turn.

3.3.7 Save the three files that are generated.

4. Data conversion and spatial registration

4.1 Select the three files into the NIRS-SPM to achieve the real coordinates registration into MNI space[28]. Affine transform the reference points and five electrode points in participants to the corresponding points in each entry according to the MRI database in MNI space.

4.2 Register the data to anatomical automatic labels and Brodmann areas and register the spatial information of the five electrode points to both of these.

4.3 Compare the coordinates of stimulation in previous research with the obtained coordinates[20,29].

4.4 Make a small cut aligned to the five points marked on the cap, such that the plastic casing is embedded snugly into the cap.

5. Stimulation

5.1. Make sure that participant have no contraindications (i.e., history of neurological or psychiatric disorders) for HD-tDCS[1,3] and that they provided written informed consent prior to the study (including HD-tDCS stimulation).

5.2 For device installation, ensure that all the necessary materials are available (**Figure 3**). Install the device as detailed in published literature[14]. A brief description is provided below.

5.2.1 Install the batteries and check that they are charged.

5.2.3 Connect the conventional tDCS and 4x1 Stimulation Adapter.

5.2.3 Connect the cables of five Ag/AgCI sintered ring electrodes to the matching receivers on the 4x1 adapter output cable.

5.2.4. Check that all materials are connected correctly.

5.3 Measure the head of the participant and place the cap on the head.

5.3.1 Embed the five plastic HD casings in the swimming cap.

5.3.2 Localize the Cz, Fpz, and Oz of the subject[13,14]. Adjust the reference on the cap to align with the international 10-10 system for scalp locations[22]. Once the cap is in position, ensure that it does not move.

5.3.3 Collect the position data of the stimulated brain areas using the 3D digitizer. Make the corresponding adjustments according to the generated data.

5.4 Cover the scalp surface with electrically conductive gel. First, carefully separate the hair through the opening of the plastic casing using the end of a plastic syringe, until the scalp is exposed. Then, cover the exposed scalp with the electrically conductive gel through the plastic casing opening on the scalp surface.

5.4 Set the parameters of the tDCS device: quality value, stimulus duration, intensity, and condition setting.

5.4.1 Turn on the 4x1 Multichannel Stimulation Adapter.

5.4.2 Ensure that the default setting is **SCAN**, which shows the impedance of one electrode at a time in the display window by scanning the electrodes[14,30,31]. Here, the impedance is described as "quality value". Values below 1.5 indicate sufficient quality[14,30,31]. In this case, the values were lower than 1.

NOTE: If the impedance value exceeds these required limits, open the cap of the plastic casing with high impedance and adjust the hair and electrode to obtain the desired impedance value.

5.4.3 Press the "**MODE SELECT**" button and switch from "**SCAN**" to "**PASS**", after the impedance values are acceptable.

5.4.4 Select the center-anode or center-cathode by pressing the "**POLARITY**" button. "**CENTRAL ANODE**" is the default setting.

5.4.5 Adjust the settings on the conventional tDCS device to include stimulus duration (min), intensity (mA) and sham condition setting. In this case, anodal active stimulation was 1.5 mA, and the stimulus lasted 20 min. Next, push the "**RELAX**" lever to switch to full current.

5.4.6 Once everything is set, initiate the stimulation. Press the "**START**" button, and the DC intensity will ramp up until the target current is reached. The timer will then show the remaining time.

NOTE: Some participants may feel uncomfortable during periods of increased DC intensity. In such cases, the current may be moderately decreased slightly for a few seconds by pulling down the "**RELAX**" lever. Then, push the dolly bar to full current, gradually, when participants feel comfortable again.

6 Post-stimulation

6.1 When the stimulation is over, turn the lever slowly to adjust the current to zero before turning off the power. Otherwise, participants may perceive stinging sensation or dizziness when turning off the power directly.

6.2 After the stimulation, open the plastic cap and remove the Ag/AgCI sintered ring electrodes from the casing.

6.3 Remove the swimming cap and clean the materials. Provide participants with tools to clean their hair.

6.4 Ask participants to fill out a questionnaire after each stimulation session, if necessary (e.g., to measure adverse effects of screening following HD-tDCS, participant tolerance to brain stimulation, etc.; see **Supplementary File**).

REPRESENTATIVE RESULTS:

Using the methods presented, coordinates of the rTPJ were determined, which requires stimulation points beyond the 10-20 system. First, the circumference of the headform should be similar to the actual head. Here, the length of the nasion to inion of the headform was ~36 cm, and the length between the bilateral preauricular was ~37 cm.

The steps for producing the electrode cap guide the measuring positions of the 10-20 system. Here, Nz, Iz, Cz, Fpz, Oz, Pz, T8, T7, C4, P8, O2, P4, C6, P6, and CP6 were determined. The approximate location of the RTPJ (about the midpoint between CP6 and P6) was found on the scalp. The distance between the central and peripheral electrodes should be adjusted based on experimental objectives. Previous research obtained radius values ranging from 3.5–7.5 cm[11,14,30]. With different radius values, DC intensity and stimulation duration may generate different electric field strengths. In this protocol, the distance between all return electrodes and the central active electrode were fixed to 3.5 cm.

Several important reference points on the swimming cap were kept, including Fpz, Cz, Oz, T8, and C4. The Vertex on the scalp was located before the stimulation, and it is critical that the Cz point on the cap exactly aligns with the Vertex. Once the cap is in position, the cap should not move. One .mat file and two .csv files after digitizing were obtained (i.e., sub01_origin.csv, which included the coordinate information of the reference [with subject number 01]), while sub01_others.csv included the coordinate information of the five targeted points [with subject number 01)].

Three .txt files were obtained after data conversion and spatial registration. In digitizer software, there are transmitter, detector (receiver), and channel options for fulfilling the requirements of fNIRS experiments. The coordinate data of the transmitter, detector, or channel should be the same. However, small operating errors may occur, because of laboratory personnel skills, pen holding gesture, etc.

Using the NIRS-SPM stand-alone registration function, the spatial registration function generates MNI coordinates. The numbers in the first line in **Table 1** represent the order in the digitizer. In this protocol, the data from number five is the position information about the

center electrode. In Brodmann areas (BA), the anatomical label and its number were obtained. The number after each line indicates the percentage of overlap. In anatomical automatic labels (AAL), the anatomical label and percentage of overlap were obtained. To reduce measurement errors, the average value of three data points from the five electrodes' final MNI coordinates were calculated. As for AALs and BAs, the value represents a percentage of overlap with the cerebral cortex. All possibilities were combined into final data (**Table 1**).

According to the data from MNI coordinates, AALs, and BAs, if the difference between the value and target value is too large, the swimming cap must be adjusted to the relative position of the actual values of X, Y, Z, and the target value, as explained in sections 2–4[11,14,30,31].

FIGURE AND TABLE LEGENDS:

Figure 1: Steps for creating the holding electrode cap.

Figure 2: 3D digitizer. The 3D digitizer is a cost-effective solution for 3D digitizing. It is a dual sensor motion tracker. The source is a magnetic transmitter that emits an electromagnetic dipole field. The sensor is a receiver that detects the field. The stylus allows accurate pinpointing of X, Y, and Z data points. The control box connects to the computer and transfer data.

Figure 3: Necessary materials for stimulation. These materials include a tDCS device, 4x1 Multichannel Stimulation Adapter, four 9 V batteries, five Ag/AgCI sodium ring electrodes, five HD plastic casings and their respective caps, electrically conductive gel, a syringe, a standard tape measure, and a swimming cap.

Table 1: Localization of stimulations in the brain area.

DISCUSSION:

Compared to traditional tDCS, HD-tDCS increases the focality of stimulation. Typical sites of stimulation are often based on the 10-20 EEG system. However, determining the precise stimulation points beyond this system can be difficult. This paper combines a 3D digitizer with HD-tDCS to determine stimulation points beyond the 10-20 system. It is important to clearly define the steps and precautions for making and using the electrode cap in such cases.

In general, the position of target stimulation areas is derived from the results of previous brain imaging studies, and the position of the stimulation areas on 10-20 international system or MNI coordinates can be obtained. The steps for creating the electrode cap guide for measuring positions of the 10-20 system are critical. It is key that the reference on the cap aligns with the international 10-20 system for scalp locations when placing the cap on the head. Once the 3D digitizer starts running, the source and sensor should not move, or it will cause data deviation.

In the software, the reference points are on the scalp and not on the cap, unless all the reference points of scalp and cap are matching. If the error between the measured results and target values is out of the acceptable range, the position of the marked points should be slightly adjusted. After adjustment, measurements should then be made again. Once users press the "MODE SELECT" button and switch from "SCAN" to "PASS", the current will start passing from the conventional tDCS device through the electrodes into the 4x1 Multichannel Stimulation Adapter. After stimulation, if the position data of the stimulated brain areas of each participant needs to be collected, it is important not to move or remove the swimming cap.

The modular electroencephalogram recording cap provides fixed positions of probes. However, determining the precise stimulation points beyond this system can be difficult. The positions of electrodes beyond the 10-20 system can be determined using the protocol described, as well as the coordinates of stimulation points. The radius setting should be based on the experimental objectives. Using the method described here, the radius of the four return electrodes and center electrode can be flexibly adjusted.

There are many digitizer software packages (e.g., the Brainstorm software for an fNIRS task; here, the Vpen software was used)[15]. Different data collection software packages emphasize different functions and should be selected according to the research question. Head circumference varies among individuals; hence, using the same cap can produce errors. However, the modular electroencephalogram recording cap also suffers from this problem.

ACKNOWLEDGMENTS:

This study was supported by the National Natural Science Foundation of China (31972906), Entrepreneurship and Innovation Program for Chongqing Overseas Returned Scholars (cx2017049), Fundamental Research Funds for Central Universities (SWU1809003), Open Research Fund of the Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences (KLMH2019K05), and the Research Program Funds of the Collaborative Innovation Center of Assessment toward Basic Education Quality at Beijing Normal University (2016-06-014-BZK01, SCSM-2016A2-15003, and JCXQ-C-LA-1). We would like to thank Prof. Ofir Turel for his suggestions on the early draft of this manuscript.

DISCLOSURES:

The authors have nothing to disclose.

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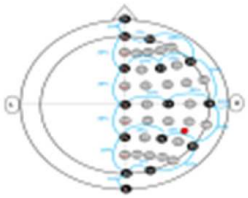
Figure 1



Determining the Stimulation Location

Mark the Stimulation Points on the Cap

Measure these Points Using 3D Digitizer

Data Conversion and Spatial Registration

Not Match

The Location of Setup
VS
The Target Location

Match

Make a Small Cut

Done

Figure 2

Click here to access/download;Figure;Figure2.eps

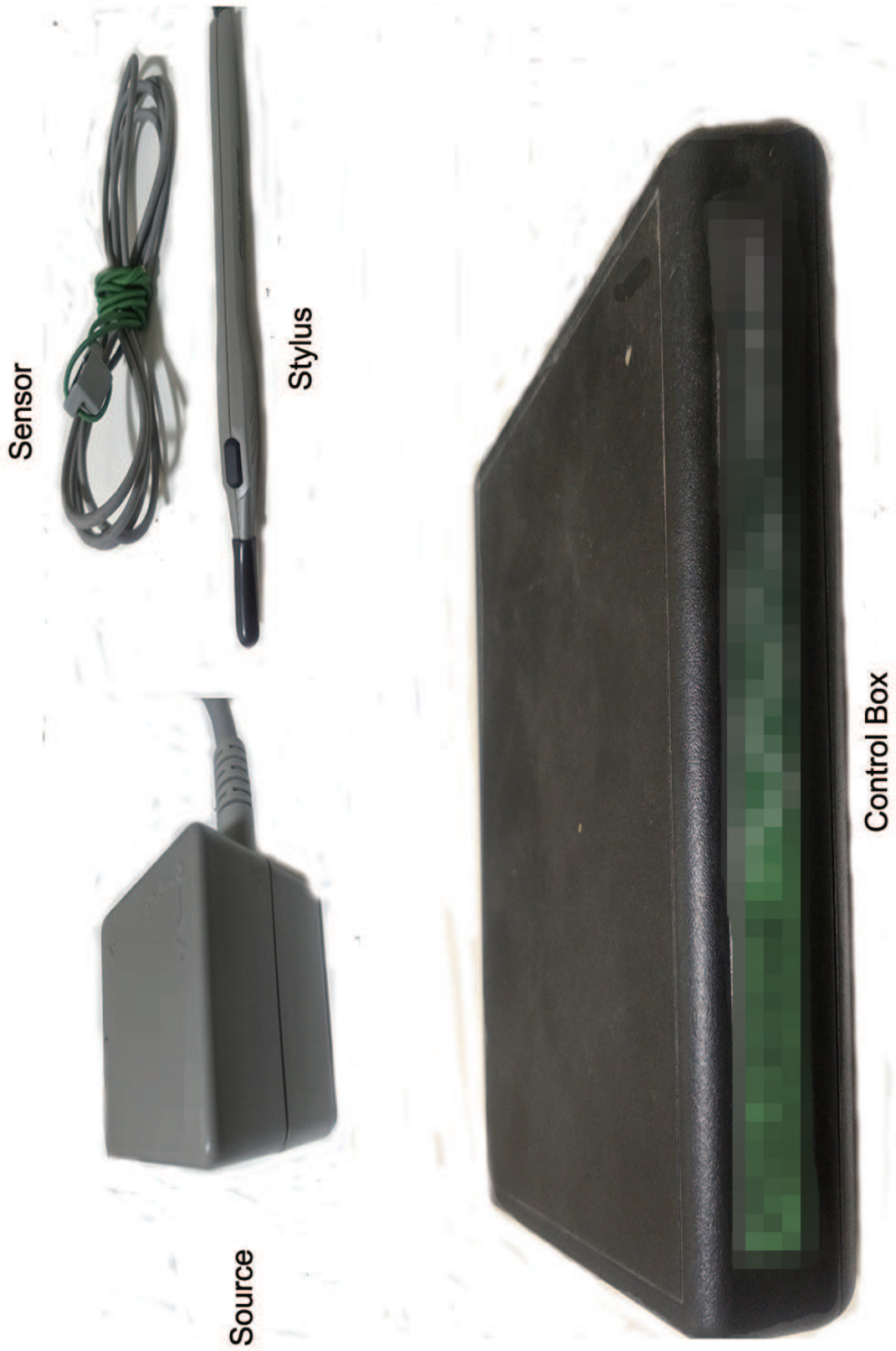


Figure 3

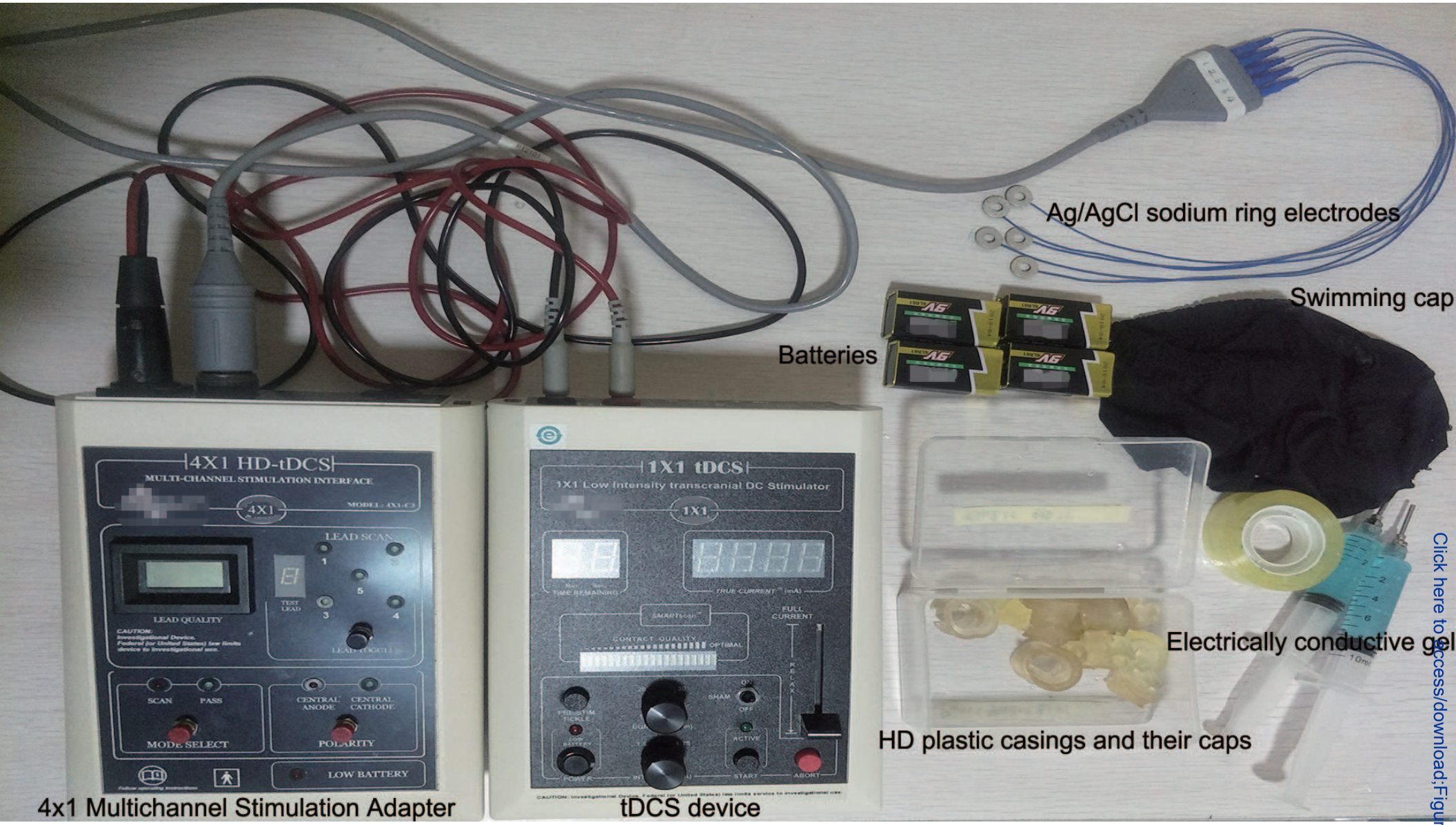


Table 1. Localization of stimulation brain area.

		1			2			
		X	Y	Z	X	Y	Z	X
MNI	Channel	43	-89	13	46	-64	54	71
	Transmit	42	-89	18	42	-67	55	71
	Receiver	43	-89	16	45	-67	54	71
	Mean	42.7	-89	15.7	44.3	-66	54.3	71
BA	Channel	**18** - Visual Association Cortex (V2), 0.27823 **19** - V3, 0.72177			**7**-Somatosensory Association Cortex, 0.27876 **39** - Angular gyrus, part of Wernicke's area, 0.53982 **40** - Supramarginal gyrus part of Wernicke's area, 0.18142			**2** –Primary So 0.41667 **22** - Superior 0.28086 **40** - Suprama Wernicke's ar **48** - Retrosub
	Transmit	**18** - Visual Association Cortex (V2), 0.15936 **19** - V3, 0.84064			**7** - Somatosensory Association Cortex, 0.57466 **39** - Angular gyrus, part of Wernicke's area, 0.34389 **40** - Supramarginal gyrus part of Wernicke's area, 0.081448			**2** - Primary So 0.38871 **22** - Superior 0.15674 **40** - Suprama Wernicke's ar **48** - Retrosub
	Receiver	**18** - Visual Association Cortex (V2), 0.21514 **19** - V3, 0.78486			**7** - Somatosensory Association Cortex, 0.42601 **39** - Angular gyrus, part of Wernicke's area, 0.51121 **40** - Supramarginal gyrus part of Wernicke's area, 0.06278			**2** - Primary So 0.44025 **22** - Superior 0.14151 **40** - Suprama Wernicke's ar **48** - Retrosub
AAL	Channel	Occipital_Mid_R, 1			Parietal_Sup_R, 0.030973 Parietal_Inf_R, 0.31416 Angular_R, 0.65487			SupraMargin Temporal_Su
	Transmit	Occipital_Mid_R, 1			Parietal_Sup_R, 0.20814 Parietal_Inf_R, 0.20362 Angular_R, 0.58824			SupraMargin Temporal_Su
	Receiver	Occipital_Mid_R, 1			Parietal_Sup_R, 0.044843 Parietal_Inf_R, 0.20179 Angular_R, 0.75336			SupraMargin Temporal_Su

3		4			5		
Y	Z	X	Y	Z	X	Y	Z
-29	25	64	-56	-16	60	-66	24
-32	27	64	-57	-16	60	-66	24
-31	27	65	-58	-12	58	-69	22
-30.7	26.3	64.3	-57	-14.7	59.3	-67	23.3
ɔmatosensory Cortex, Temporal Gyrus, ırginal gyrus part of rea, 0.19136 ɔicular area, 0.11111		**20** - Inferior Temporal gyrus, 0.089606 **37** - Fusiform gyrus, 0.91039			**21** - Middle Temporal gyrus, 0.0072464 **22** - Superior Temporal Gyrus, 0.17391 **37** - Fusiform gyrus, 0.07971 **39** - Angular gyrus, part of Wernicke's area, 0.73913		
ɔmatosensory Cortex, Temporal Gyrus, ırginal gyrus part of rea, 0.31034 ɔicular area, 0.1442		**20** - Inferior Temporal gyrus, 0.035842 **37** - Fusiform gyrus, 0.96416			**21** - Middle Temporal gyrus, 0.0072464 **22** - Superior Temporal Gyrus, 0.17391 **37** - Fusiform gyrus, 0.07971 **39** - Angular gyrus, part of Wernicke's area, 0.73913		
ɔmatosensory Cortex, Temporal Gyrus, ırginal gyrus part of rea, 0.28302 ɔicular area, 0.13522		**20** - Inferior Temporal gyrus, 0.0071429 **37** - Fusiform gyrus, 0.99286			**19** - V3, 0.0036101 **22** - Superior Temporal Gyrus, 0.054152 **37** - Fusiform gyrus, 0.12274 **39** - Angular gyrus, part of Wernicke's area, 0.81949		
al_R, 0.65741 ıp_R, 0.34259		Temporal_Mid_R, 0.039427 Temporal_Inf_R, 0.93907 Cerebelum_Crus1_R,0.021505			Occipital_Mid_R, 0.13406 Angular_R, 0.33696 Temporal_Sup_R,0.032609 Temporal_Mid_R, 0.49638		
al_R, 0.74922 ıp_R, 0.25078		Temporal_Mid_R, 0.032258 Temporal_Inf_R, 0.94265 Cerebelum_Crus1_R, 0.02509			Occipital_Mid_R, 0.13406 Angular_R, 0.33696 Temporal_Sup_R,0.032609 Temporal_Mid_R, 0.49638		
al_R, 0.7673 ıp_R, 0.2327		Temporal_Mid_R, 0.11429 Temporal_Inf_R, 0.88571			Occipital_Mid_R, 0.22022 Angular_R, 0.15523 Temporal_Mid_R, 0.62455		

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
1X1 Low Intensity transcranial DC Stimulator	Soterix Medical	1300A	
3-dimensional Polhemus-Patriot Digitizer	POLHEMUS	1A0453-001	PATRIOT system component
4X1 Multi-Channel Stimulation Interface	Soterix Medical	4X1-C3	
Dell desktop computer	Dell	CRFC4J2	Master computer to run 3D digitizer application

Response Letter

Dear Editor,

We would like to thank you and the review team for the constructive suggestions. We have carefully revised the manuscript based on these suggestions and we re-submit this revision for your consideration. Point by point responses to the reviewers' comments are provided below, in blue font.

We hope that the revised version of the manuscript is now acceptable for publication in JoVE.

We look forward to hearing from you soon.

Yours sincerely,

Qinghua He on behalf of all authors

heqinghua@gmail.com; heqinghua@swu.edu.cn.

Response to Editor

1. The editor has formatted the manuscript to match the journal's style. Please retain the same.

Response: Thank you. We retain the journal’s format in the revision.

2. Please address specific comments marked in the attached manuscript.

Response: We appreciate these suggestions. We have rewritten the related parts and provided more details in the abstract and protocol sections. In addition, we added a Supplementary Material file that provides more details and specifics.

How many participants were used for the study? In how many participants were these new stimulation points determined? Some statistical analysis if done.

Response: Thank you for these constructive comments. In the preparation of electrode holding cap, we collected the stimulation position data of one participant three times repeatedly, in order to reduce error. During the stimulation procedure, we collected the position data of the stimulated brain areas of each participant. We made the corresponding adjustments according to the individually generated data before stimulation. This improves the stimulation accuracy for each individual.

3. Once done, please proofread the manuscript for any grammar or spelling errors.

Response: Thank you. We double checked the text and revised the manuscript carefully. The revised version has been proofread; it should be easier to follow and free of typos.

4. Please highlight 2.75 pages of the protocol including headings and spacing for filming purpose.

Response: Thank you for this comment. In response, we highlight the essential steps of the protocol for the video in yellow.

Response to Reviewer 3

1. Describe the name of the tDCS device used, the advice with running the machine will vary depending on the make/model. Information is also missing in regards to the software recommended to use with the digitiser.

Response： Thank you for this comment. The use of such names is not allowed in the manuscript. Nevertheless, we provide the requested information in the Materials file.

2. The claim that the paper goes beyond the 10-20 system seems to be misleading to me.

The method describe is still heavily based on this system. For concerns with the accuracy (and reproducibility) of tDCS electrode placement, it would be nice to see a study that directly compares the accuracy of multiple techniques and how these affect behavioural outcomes E.g., placement via 10-20 alone, with the addition of a 3D digitiser, using a neuro-navigation system with a standard head model, and using a neuro-navigation system with individual MRI scans. IN the least, can the authors provide any evidence/justification that the method they describe is more accurate? Especially given it requires more equipment/cost than the 10-20 system alone. And, related, some indication of cost?

Response：Thank you for these constructive comments. We changed the related statements to be more objective and accurate. The finite electrode points of 10-20/10-10 system do not cover all cortical areas. Moreover, we are still not sure if the stimulation points are the targeted cortical areas by placement via 10-20/10-10 alone. The method with the addition of a 3D digitizer can overcome the abovementioned limitation. Previous studies used this method to obtain the probability that the stimulation points correspond to cortical areas[1-3]. According to the generated value, we can make the needed adjustments to improve the accuracy.

To use broadly and conveniently, 3D digitizer is cost effective and is suitable for use with other techniques including the functional Near-Infrared Spectroscopy (fNIRS), EEG and so on.

3. Provide a reference for point 5.1., so potential researchers can look up subject safety guidelines for tDCS.

Response：We appreciate this suggestion. In response, we added the reference for point 5.1.

Response to Reviewer 4

Major Concerns:

1. the title and introduction are not clear and easy to read.

Response: Thank you for this comment. We changed the related statements to be more objective and accurate. The title now reads:" Determining more Precise Stimulation Location by Combining a 3D Digitizer with High-Definition Transcranial Direct Current Stimulation (HD-tDCS) ".

2. some concepts are very superficial with respect to the way tDCS works. For example:

" It aims at helping psychiatric patients and at establishing causality between neural excitability and behavior in healthy people"

" Neuronavigation is the technique that allows for mapping the interaction between transcranial magnetic stimulation (TMS) and the human brain"

Response: Thank you. We changed the related paragraphs. They now provide more detail. For example, we write that:

” Transcranial direct current stimulation (tDCS) is a non-invasive technique that modulates cortical excitability with weak direct currents over the scalp. It aims at establishing causality between neural excitability and behavior in healthy people[1-3]. In addition, as a motor neurorehabilitation tool, tDCS is widely used in the treatment of Parkinson’s disease, stroke and cerebral palsy[4].”.

“Neuronavigation is the technique that allows for mapping the interaction between transcranial stimulation and the human brain. Its visualization and three-dimensional image data are used for precise stimulation.”.

3. As opposed to what Authors claim, MRI is usually essential for neuronavigation and often time there is no point in neuronavigating without MRI.

Response: Thank you for this comment. We agree with that MRI is usually essential for neuronavigation. However, some participants (e.g., with a metal implant, claustrophobia, pregnant women, etc.) cannot be easily scanned with MRI techniques. The method we applied does not require MRI scans and is based on the vast data from the structural

images to overcome the abovementioned limitations. That is to say, we still rely on structural images produced by MRI to determine more precise stimulation, compared to when not using MRI or our technique.

4. An important aspect is quite neglected, which is the interaction between tDCS electric field and head structures.

Response: Thank you for this constructive comment. Previous studies found that highly conductive cerebro-spinal fluid (CSF) lead to poor focal stimulation of tDCS[4]. However, using the multiple small electrodes and systematically optimizing the currents can achieve targeted stimulation[5]. Compared to conventional tDCS, the 4 x 1 HD-tDCS has diminished electrode size, and it reduces affected cortical area size, which results in more spatially restricted electric field. Moreover, in this protocol, our emphasis is on the accuracy of electrode placement. Therefore, there is not much discussion about this issue.

5. If the Authors believe that there is no point in considering head MRI because tDCS effects are very broad, then this should be claimed and supported, but in this case I would rather argue that perhaps neuronavigation does not improve tDCS targeting. Conversely, if the Authors believe that HD-tDCS has very focal effects, there is no other way than using a personalized brain MRI.

Response: Thank you for these comments. A key limitation of tDCS is the difficulty in precisely focusing the stimulating electric field[6]. Compared to tDCS, HD-tDCS employs

the small ring of electrodes to diminish electrode size, which results in more focal stimulation of selected brain regions[7]. However, a common assessment of the site of stimulation on the scalp has usually been the EEG 10-20 system. This approach limits the placement of the electrode points, and we cannot verify whether the placement is on the targeted cortex. The best way to get a more exact probe position is using a personalized brain MRI. But when participants cannot scan in MRI (due to health or cost issues), the method we applied is also a good way to overcome the abovementioned limitation.

6. The procedure basically describes the use of a neuronavigation system, and I am not sure whether this is specific to HD-tDCS.

Response: Thank you for this comment. The method is also suitable for use with other techniques to verify the location of specific brain regions (For example, functional Near-Infrared Spectroscopy, fNIRS)[8].

7. Some specific wording which may change depending on the commercial products is reported in the manuscript. I wonder if this could be better handled.

Response: Thank you. We provide the commercial information of products in the Materials file for reference. In this protocol, we mainly provide a way to combine

neuronavigation with non-invasive brain stimulation to improve the accuracy of stimulating location. This method is suitable for other digitizers, but the operation may be different. Moreover, different commercial products should be selected as per the research questions. Hence, we keep our description product-free,

Reference

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Adverse effect screening for HD-tDCS*

1. How much you perceive the stimulation to affect your behavior?
   1 (not at all) 2 3 4 5 6 7 (extremely)

2. Do you think electrical stimulation is acceptable for you?
   1 (can't bear) 2 3 4 5 6 7 (totally acceptable)

3. Did you experience any of the following symptoms or side effects?
   (1) Headache
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (2) Neck pain
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (3) Scalp pain
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (4) Scalp burns
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (5) Tingling
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (6) Skin redness
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (7) Sleepiness
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (8) Trouble concentrating
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (9) Acute mood change
   Do you think this is related to HD-tDCS ? 1 (none) 2 3 4 5 (extreme)

   (10) Other (Please Specify):

* Participants were asked to fill out the questionnaire after the stimulation for screening the adverse effect.

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Author(s):	Wanting Chen, Rui Chen, and Qinghua He

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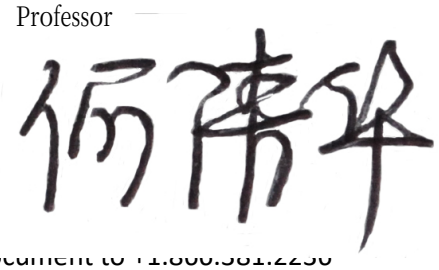
Signature:	
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