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Corresponding Author:	Adrianna Giuffre University of Calgary Calgary, AB CANADA
Corresponding Author's Institution:	University of Calgary
Corresponding Author E-Mail:	Adrianna.Giuffre@albertahealthservices.ca
Order of Authors:	Adrianna Giuffre
	Lauran Cole
	Hsing-Ching Kuo
	Helen L. Carlson
	Jeff Grab
	Adam Kirton
	Ephrem Zewdie
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TITLE:

Non-Invasive Modulation and Robotic Mapping of Motor Cortex in the Developing Brain

AUTHORS & AFFILIATIONS:

Adrianna Giuffre[1,2], Lauran Cole[1,2,3], Hsing-Ching Kuo[4,5], Helen L. Carlson[2,3,4,5], Jeff Grab[6], Adam Kirton[2,3,4,5,7], Ephrem Zewdie[3,4]

[1] Department of Neurosciences, University of Calgary, Calgary, AB, Canada
[2]Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada
[3]Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[4] Department of Pediatrics, University of Calgary, Calgary, AB, Canada
[5] Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB, Canada
[6] Faculty of Medicine and Dentistry, University of Alberta, AB, Canada
[7] Department of Clinical Neurosciences, University of Calgary, AB, Canada

Corresponding Author:
Adam Kirton
Email Address: adam.kirton@ahs.ca

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SHORT ABSTRACT:

We demonstrate protocols for the modulation (tDCS, HD-tDCS) and mapping (robotic TMS) of the motor cortex in children.

LONG ABSTRACT:

Mapping the motor cortex with transcranial magnetic stimulation (TMS) has potential to interrogate motor cortex physiology and plasticity but carries unique challenges in children. Similarly, transcranial direct current stimulation (tDCS) can improve motor learning in adults but has only recently been applied to children. The use of tDCS and emerging techniques like high–definition tDCS (HD-tDCS) require special methodological considerations in the developing brain. Robotic TMS motor mapping may confer unique advantages for mapping, particularly in the developing brain. Here, we aim to provide a practical, standardized approach for two integrated methods capable of simultaneously exploring motor cortex modulation and motor maps in children. First, we describe a protocol for robotic TMS motor mapping. Individualized, MRI-navigated 12x12 grids centered on the motor cortex guide a robot to administer single-pulse TMS. Mean motor evoked potential (MEP) amplitudes per grid point are used to generate 3D motor maps of individual hand muscles with outcomes including map area, volume, and center of gravity. Tools to measure safety and tolerability of both methods are also included. Second, we describe the application of both tDCS and HD-tDCS to modulate the motor cortex and motor learning. An experimental training paradigm and sample results are described. These methods will advance the application of non-invasive brain stimulation in children.

INTRODUCTION:

Non-invasive brain stimulation can both measure and modulate human brain function[1,2]. The most common target has been the motor cortex, due in part to an immediate and measurable biological output (motor evoked potentials) but also the high prevalence of neurological diseases resulting in motor system dysfunction and disability. This large global burden of disease includes a high proportion of conditions affecting children such as cerebral palsy, the leading cause of lifelong disability affecting some 17 million persons worldwide[3]. Despite this clinical relevance and the diverse and increasing capacities of neurostimulation technologies, applications in the developing brain are only beginning to be defined[4]. Improved characterization of existing and emerging non-invasive brain stimulation methods in children are required to advance applications in the developing brain.

Transcranial magnetic stimulation (TMS) is a well-established neurophysiological tool being increasingly used for its non-invasive, painless, well-tolerated and safety profile in adults. TMS experience in children is relatively limited but steadily increasing. TMS delivers magnetic fields to induce regional activation of cortical neuronal populations in the brain with net outputs reflected in target muscle motor evoked potentials (MEP). Systematic application of single pulse TMS can define maps of the motor cortex in vivo. Seminal animal studies[5] and emerging human TMS studies[6] have shown how motor maps may help inform mechanisms of cortical neuroplasticity. Navigated motor mapping is a TMS technique that is used to map out the human motor cortex to interrogate functional cortical regions. Changes in motor map have been associated with plastic changes of the human motor system[7]. Recent advancements in robotic TMS technology have brought new opportunities to improve motor mapping efficiency and accuracy. Our group has recently demonstrated that robotic TMS motor mapping is feasible, efficient, and well tolerated in children[8].

Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation that can shift cortical excitability and modulate human behaviors. There has been a multitude of studies examining the effect of tDCS in adults (>10,000 subjects) but less than 2% of studies have focused on the developing brain[9]. Translation of adult evidence to pediatrics applications is complex, and modified protocols are needed due to complex differences in children. For example, we and others have shown that children experience larger and stronger electric fields compared to adults[10,11]. Standardization of tDCS methods in children is important to ensure safe and consistent application, improve replication, and advance the field. Experience of motor learning modulation tDCS in children is limited but increasing[12]. Translational applications of tDCS to specific cerebral palsy populations are advancing towards late phase clinical trials[13]. Efforts toward more focal stimulation applied through high-definition tDCS (HD-tDCS) has only just been studied for the first time in children[14]. We demonstrated that HD-tDCS produces similar improvements in motor learning as conventional tDCS in healthy children[14]. Describing HD-tDCS methods will allow for replication and further applications of such protocols in children.

PROTOCOL:

All the methods described in this protocol have been approved by Conjoint Health Research Ethics Board, University of Calgary (REB16-2474). The protocol is described in **Figure 1**.

1. Non-invasive brain stimulation contraindications

1.1. Screen all participants for contraindications for TMS[15] and tDCS[1] prior to recruitment.

2. Transcranial magnetic stimulation motor mapping

2.1. Preparing MRI for navigated TMS

2.1.1. Obtain each participant's structural MRI (T1). If an MRI is unobtainable, use a template MRI from Montreal Neurological Institute.

2.1.2. Import the MRI file in DICOM or NIFTI format to the neuronavigation software (see **Table of Materials**).

2.2. TMS target trajectories

2.2.1. Use the neuronavigation software to reconstruct **Skin** and **Full Brain Curvilinear** using the tabs.

2.2.2. Select **New**, **Skin**, and **Compute Skin**. Ensure the nose and top of the head are included.

2.2.3. Select **New**, and **Full Brain Curvilinear**. Enclose the green selection box outside of the brain but inside of the skull. Select **Compute Curvilinear**. Adjust the peel depth to 4.0-6.0 mm.

2.2.4. Select **Configure Landmarks**. Place four landmarks at the tip of nose, nasion, and the notches of both ears of the reconstructed skin. Name the landmarks corresponding to their anatomy.

2.2.5. Select the **Targets** tab to view curvilinear brain. Select **New**, and **Rectangular Grid**. Place uniform 12 x 12 coordinate grids with 7 mm spacing on the surface of the reconstructed brain over the "handknob" of the motor cortex (precentral gyrus)[17].

2.2.6. Use the **Target Positioning Tool** on the right to optimize the grid positioning for rotation, tilt, and curvature. Convert the grid-points into trajectories that will guide the robot to position the TMS coil. Adjust the angle of the trajectories so they are 45° to the longitudinal fissure of the brain.

2.2.7. Use the **SNAP** tool to extrapolate and optimize the trajectories to curvilinear brain.

2.2.8. Initialize and position the TMS robot arm and seat to **Welcome position** and calibrate the force plate sensor using **Force sensor test**.

2.3. Preparing the participant for motor mapping

2.3.1. Have the participants fill out a safety questionnaire[18].

2.3.2. Once the participants seated comfortably in the robot chair, adjust the backrest and neckrest, and ensure their feet are supported. Support their arms and hands with pillows to ensure their hands are in a resting position for the duration of the mapping session.

NOTE: Children and adolescents will need reminders throughout the session to keep their hands relaxed.

2.3.3. Clean the skin over the muscle of interest. Place Ag/AgCl surface electrodes on both hands and forearms of the participant, targeting four distal forelimb muscles, 1) the belly of the first dorsal interosseous (FDI), 2) abductor pollicis brevis (APB), 3) abductor digiti minimi (ADM), and 4) the wrist extensor (extensor carpi ulnaris).

2.3.4. Connect the surface electrodes with electromyography (EMG) amplifier and data acquisition system and connect the amplifier to a data collecting computer with a compatible EMG software.

2.3.5. Co-register the four landmarks on the head of the participant using the landmark pointer. Use the validation tab to ensure the participant's head is properly registered.

2.4. Determining motor mapping TMS intensity

2.4.1. Select a grid-point closest to the participant's "handknob". Select the **Align to Target** button to align the TMS coil held by the robot to this target location. Select **Contact on**. Monitor the contact quality using the contact force indicator. Ensure the indicator is green or yellow.

NOTE: The red color on the contact indicator means there is too much force on the participant's head. No color means the TMS coil is not in contact with the participant's head. In these cases, adjust the force plate sensitivity.

2.4.2. Instruct the participant not to move outside the scope of the robot arm. Ensure the participant's hand muscles are relaxed and remain still prior to contact.

2.4.3. Select **Align and Follow** so the coil remains centered on the target if the participant moves.

2.4.4. Use the TMS trigger button on the TMS machine to deliver 5-10 TMS pulses at an intensity between 40-60% maximum stimulator output (MSO). Repeat this step to 5-6 grid-points surrounding the "handknob".

2.4.5. Determine the grid-point that gives the largest and most consistent (hotspot) motor evoked potential (MEP) for the left or right FDI muscle.

2.4.6. Determine the Resting Motor Threshold (RMT) as the lowest intensity that produces an MEP of at least 50 µV in the FDI muscle in 5/10 stimulations.

2.5. Motor mapping

2.5.1. Starting from the grid-point closest to the hotspot, deliver four single-pulse TMS pulses (1 Hz) at an interstimulus of 1 s and TMS intensity of 120% RMT. A responsive grid-point is determined by 2/4 MEPs >50 µV in any of the hand muscles.

2.5.2. Move to the adjacent grid-point and repeat the above step.

2.5.3. Continue sequentially in a linear fashion along responsive points until a non-responsive point is reached, which is the first border region of the map.

2.5.4. Continue mapping to establish the border points in all four directions of the rectangular grid.

2.5.5. Record all MEPs from all muscles using the EMG software for offline analysis.

2.5.6. After 3-4 grid points, select **Contact off** and give the participant a break until they feel ready to continue.

2.5.7. Throughout the mapping session, continuously check in with the participant to ensure they are comfortable and/or need a break.

2.5.8. Use a hard copy version of the same grids to tack the simulation order for further analysis.

2.5.9. Complete mapping using a robotic TMS as described here or manually (not described in this manuscript). If using a TMS Robot, it will move to the grid-point selected by the experimenter. The robot will accommodate for child head motion in near real time. This will alleviate any additional movement associated with a technician manually holding the coil on the participant's head.

NOTE: If mapping using a TMS robot, ensure there is an experimenter beside the robot at all times during the session. If the robot is placed on a participant's head and the participant suddenly moves, the robot will try to follow their head. If the participant must move, sneeze, scratch, or perform an activity involving the movement of their head, the robot arm must be moved to prevent the participant's head from hitting the robot's arm or TMS coil.

2.6. Motor map creation

2.6.1. Using a custom-made coding script, generate three-dimensional motor maps (**Figure 2**).

2.6.2. Calculate motor map area and volume using responsive trajectory sites. Calculate center of gravity (COG) as weighted average of the motor representations of each coordinate location.

NOTE: Map area is calculated as the grid spacing (7 mm)^2 multiplied by the total number of responsive sites. Map volume is calculated as the cumulative sum of grid spacing multiplied by the mean MEP amplitude at each responsive site. A user-friendly version of the script is being developed to share with the public as open source. Meanwhile, contact the corresponding author to get access to the script.

3. Conventional tDCS and HD-tDCS application

3.1 Randomize the participants to one of three intervention groups (sham, conventional tDCS, HD-tDCS).

3.2 Have the participant complete the Purdue Pegboard Test (PPT) three times using their left hand (non-dominant), establishing their baseline score.

3.3 Inspect electrode quality to confirm the integrity of the tDCS sponge inserts and rubber electrodes.

3.4 Turn on the conventional tDCS device by flipping the power switch to **ON**.

NOTE: Ensure the low battery light is not illuminated. If it is illuminated, change the batteries before starting the session.

3.4.1 For participants receiving conventional or sham tDCS, lightly soak two 25 cm^2 sponge electrodes with saline. Ensure the entire electrode is covered but not dripping. Insert the rubber electrode into the saline soaked sponge electrodes and connect each electrode to the tDCS device.

3.5 Locate the marked hotspot (Right M1) using the neuronavigation and mark it with a non-toxic marker. At the end of each tDCS, HD-tDCS or sham session, mark the hotspot again so that it is visible the next day.

3.5.1 If randomized to conventional tDCS or sham tDCS, place one 25 cm^2 saline-soaked sponge electrode over the participant's marked hotspot (Right M1), serving as the anode. Place the other 25 cm^2 saline-soaked sponge electrode on the contralateral supraorbital region, representing the cathode. Use a light plastic pediatric "headband" to hold electrodes in place.

NOTE: Ensure that there is no saline dripping from the electrode as it may shunt the current.

3.5.2 In the sham and conventional tDCS group, ensure "optimal" contact quality. If the contact quality is "sub-optimal", inject a small amount of saline solution under the sponge electrodes, or ensure that there is minimal hair between the scalp and electrode.

NOTE: “Optimal” contact quality is achieved when more than half of the quality of contact indicator lights are on. If less than half of the contact indicator lights are on, the contact quality is sub-optimal. Do not start stimulation if only one of two of the indicator lights are on.

3.5.3 In the HD-tDCS group, refer to Villamar, M.F., et al.[16] for the appropriate set-up.

3.5.4 In the HD-tDCS group, set the device to the **Scan** setting to check the impedance at each electrode. Ensure the impedance is under 1 “quality unit” and described previously[19,20]. If contact quality is poor, remove the electrode and check that there is no hair obstructing the contact of the electrode, and that a continuous column of electrode gel is present between the scalp and electrode. If needed, apply more electrode gel.

3.6 Set the tDCS and HD-tDCS device to the anode montage setting, 1 mA current strength, and 20 min duration.

3.7 Ensure the participant is sitting comfortably and they understand the possible sensations they may experience (such as itchy or tingling sensations). Remind the participant to communicate if they feel any discomfort or if they have any questions.

3.7.1 In the conventional tDCS and HD-tDCS groups, make sure the toggle is set to **Active**.

NOTE: For the sham group, the toggle should be set to **Sham**. This setting should be hidden from the participant.

3.7.2 Press the device’s **Start** button to start stimulation. Ensure the duration is set to 20 min, and the intensity to 1 mA.

NOTE: In the conventional tDCS and HD-tDCS groups, the current will ramp up over 30 s to 1 mA and continue for 20 min. In the sham tDCS group, current will be ramped up over 30 s to 1 mA and immediately ramped down over 30 s.

3.8 At 5 min, 10 min, 15 min, and 20 min, have the participant complete the PPT three times using their left hand.

3.9 After 20 min, turn the device off after the intensity finishes ramping down to 0 mA.

NOTE: For participants receiving either conventional tDCS or HD-tDCS, the machine will automatically ramp down to 0 mA at 20 min. For participants receiving sham tDCS, the machine will automatically ramp up over 30 s to 1 mA and immediately ramp down to 0 mA over 30 s at 20 min.

3.10 Remove the electrodes from the participant’s head.

3.11 For sham and conventional tDCS group, remove black electrodes from inside the sponges

and rinse the sponge electrode with normal tap water.

3.11.1. In the HD-tDCS group, take off the plastic electrode holder top and remove the electrodes. Remove the electrode cap from participants' head. Rinse any gel in the electrode holder. Clean the electrode with a slightly damp paper towel. Wipe the electrode with a dry paper towel to remove any remaining gel.

3.12 Have all participants complete the *Transcranial Direct-Current Stimulation Side-effects and Tolerability* questionnaire after each stimulation session.

3.13 Have the participants complete the PPT three times using their left hand.

3.13.1. Have the participants return the following day and for another four consecutive days (five days total) for non-invasive brain stimulation (sham, tDCS, or HD-tDCS) paired with motor learning (PPT). Repeat steps 3.2-3.13 on Day 2-4. On Day 5, have the participants begin with non-invasive brain stimulation (sham, tDCS or HD-tDCS) (steps 3.2-3.13 are repeated). After a break (45 min-~1.5 h since receiving stimulation), start robotic TMS motor mapping (steps 2.3-2.5.8).

NOTE: All participants received the same number of minutes for breaks between assessments.

3.13.2. After 6-weeks, invite the participants to return and perform the PPT without receiving any non-invasive brain stimulation (step 3.2 followed by robotic TMS motor mapping (step 2.5.8)).

REPRESENTATIVE RESULTS:

Using the methods presented here, we completed a randomized, sham-controlled interventional trial[8]. Right-handed children (n = 24, ages 12-18) with no contraindications for both types of non-invasive brain stimulation were recruited. Participants were specifically excluded in this study if on neuropsychotropic medication or if they were not naïve to tDCS. There were no dropouts.

Robotic TMS motor maps were obtained to acquire a baseline motor map and to serve as a potential mechanism to monitor neuroplastic and cortical excitability changes after motor learning paired with non-invasive brain stimulation. Using the methods described above, all participants received three robotic TMS motor maps, 1) baseline prior to non-invasive brain stimulation (sham, tDCS, or HD-tDCS), 2) day 5 (Post), and 3) at the 6-week follow up (retention time). All participants received bihemispheric motor mapping (3 participants received right hemispheric motor mapping only due to time constraints). Motor maps were completed on average in 18 min for unilateral motor maps and 36 min for bihemispheric mapping. Motor map area, volume, hotspot, and COG were computed and compared at the individual and group level. In our initial motor map analysis, motor map area and volume did not change significantly following the intervention. In our secondary analysis, measuring submaximal proportions of map area and volume resulted in significantly smaller variance ($p<0.05$).

All participants received one of three non-invasive brain stimulation interventions for a duration

of 20 min (1 mA) for five consecutive days. We demonstrated that tDCS and HD-tDCS improve the rate of learning (number of pegs/day) (tDCS p=0.042, HD-tDCS p=0.049) over 5 days of training. The active intervention groups (tDCS and HD-tDCS) had larger improvements in daily average left hand PPT score (PPT_L) at day 4 and 5 compared to sham (day 4 $p \leq 0.043$, day 5 $p \leq 0.05$) (**Figure 3**). The active intervention groups retained their motor skills (on the PPT) at 6-weeks post-training. However, there was significant skill decay in the sham group from post-training to the 6-week follow-up (p=0.034). This methodology has been replicated from a previous study[21] and the datasets were combined (**Figure 4**). The replication data demonstrated similar results. There was a significant increase in the rate of learning observed in the tDCS and HD-tDCS group compared to the sham group (tDCS p = 0.001, HD-tDCS p = 0.012).

FIGURE LEGENDS:

Figure 1. Trial protocol. PTT= Purdue pegboard Test, TMS= TMS motor mapping tDCS= transcranial direct current stimulation, HD-tDCS = High-definitional tDCS.

Figure 2. An example TMS motor map. Top view of left FDI motor map (**A**) Pre and (**B**) post HD-tDCS intervention. Red cross indicates hotspot, blue cross indicates COG. The color bar indicates the range of MEP from 0-2 mV.

Figure 3: Motor learning observed in sham, tDCS and HD-tDCS groups. This figure has been republished from Cole & Giuffre et al. 2018**. (A)** Mean daily change in left hand Purdue Pegboard score from baseline in sham (white triangles), tDCS (grey circles), and HD-tDCS (black circles), (*n* = 24). **(B)** Daily mean score at each time point of PPT_L. *p<0.05 for tDCS vs. sham, # p<0.05 for HD-tDCS vs. sham. Error bars indicate standard error.

Figure 4: Replication of methods - combined PPT_L dataset for 3 days of training. This figure has been republished from Cole & Giuffre et al. 2018)**. (A)** The learning curves for sham (white triangles, *n* = 14), tDCS (gray circles, *n* = 14), and HD-tDCS (black circles, *n* = 8) groups. **(B)** Mean daily learning for sham, tDCS, and HD-tDCS from the combined studies. Error bars indicate standard error.

DISCUSSION:

TMS has also been explored in clinical pediatric populations, including perinatal stroke[22] and cerebral palsy, where TMS motor maps were successfully created in children with cerebral palsy to explore mechanisms of interventional plasticity. Using an established protocol[8], TMS motor maps were successfully collected in typically developing children, and are currently being collected in an ongoing multicenter clinical trial for children with perinatal stroke and hemiplegic cerebral palsy (NCT03216837). Describing TMS motor mapping methods will allow for replication and further applications of protocols in healthy children and children with movement disorders.

Robotic motor mapping improves TMS coil placement accuracy and reduces human error when compared to manual techniques[23,24]. This technique is more advantageous for pediatric populations who have increased head movements and lower tolerability for long sessions[12]. Although motor mapping using a TMS robot has been reported in adults, our group is the first to

apply this technique in a pediatric population. New motor mapping methodologies that use statistical weighting and interpolation[25,26] can be used to decrease acquisition time if combined with robotic TMS. As such, methodologies should be further explored in the developing brain.

We outline a succinct approach to apply tDCS, HD-tDCS, and TMS in a healthy pediatric population. There are a variety of critical steps to consider in the application of non-invasive brain stimulation in children. It is crucial that children and/or their parents confirm that the participant has no contraindications for non-invasive brain stimulation. It is important for participants to feel comfortable and safe. Encourage the participants to ask questions throughout the session as it is necessary to continuously obtain feedback throughout the session, especially in a pediatric population. As well, it is important to inspect the quality of the electrodes and the quality of the participants' scalp, as this precludes safe application of tDCS. It is vital to have the correct anodal montage, current intensity, and duration of stimulation selected on the machine before starting the stimulation. There are specific considerations for conventional tDCS and HD-tDCS. In HD-tDCS, it is crucial to rotate the electrode chosen to be in the center anodal position with the surrounding electrodes to decrease the amount of electrode breakdown. It is vital to have the correct connection of the cables to the anodal and cathodal ports on the 1x1 tDCS machine in conventional tDCS to allow for the correct polarity to be applied. Previous literature has demonstrated the importance of using saline solution to improve tolerability of the stimulation[27]. The most common sensation described in our study was itching (56%)[14]. We have reported no adverse effects in our population using our methods described[12,14].

There are a variety of different modifications to make when perfecting the application of tDCS and HD-tDCS. It is important to have good contact quality to decrease the resistance of the current across the scalp. If the contact quality is poor, more saline solution can be applied to decrease the resistance in conventional tDCS. However, it is important to first ensure that good electrode contact with the scalp is present. In HD-tDCS, it is essential that the scalp be exposed to allow for better quality of electrode. Hair may need to be further brushed out of the way and more electrode gel applied to improve the contact quality. Ensure that the contact quality is continuously monitored throughout the session.

Current modeling studies have suggested a difference in current strength experienced across age groups depending on white matter and CSF volume[10,11]. A limitation of this method is that we did not perform prospective current modeling on each participant to apply a current strength that would induce comparable neuronal electric field strength across participants.

This method is an important next step in the application of non-invasive brain stimulation in pediatrics. We have extended our training period from three days to five days and observed similar improvements in skill. HD-tDCS has only been applied in a pediatric population using our method and we have demonstrated that there is similar motor skill learning to conventional tDCS. HD-tDCS induces a more focal current, improving targeting and implication[28]. The methods described in this paper will allow for the replication and further study of HD-tDCS in children.

These methods are currently being extended to a perinatal stroke population. The tDCS and HD-

tDCS protocol has been adapted to this population and training time has been extended to further develop clinical trials in perinatal stroke. It is crucial to optimize the application of tDCS in pediatrics to advance therapeutic application in children with perinatal stroke and therefore improve motor function outcomes. For TMS motor mapping, it is important to ensure that the participant is comfortably seated, with their arms and hands in a relaxed position. Following full motor mapping session, only 15% of the participants experienced mild self-limiting headache.

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DISCLOSURES:

The authors have no disclosures.

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Figure

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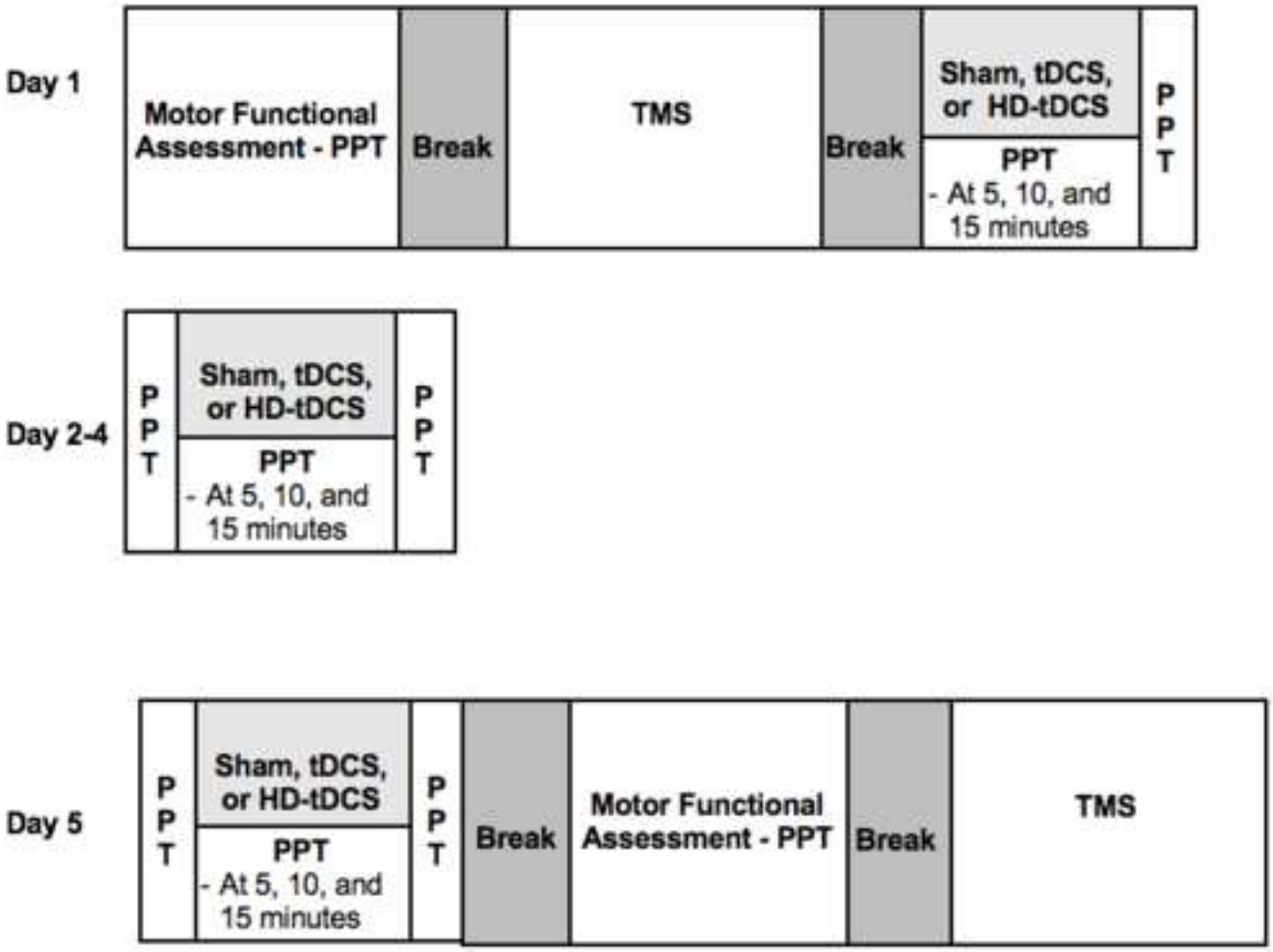
A.



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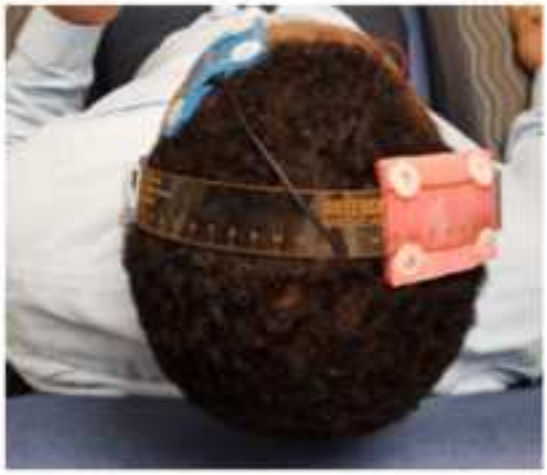

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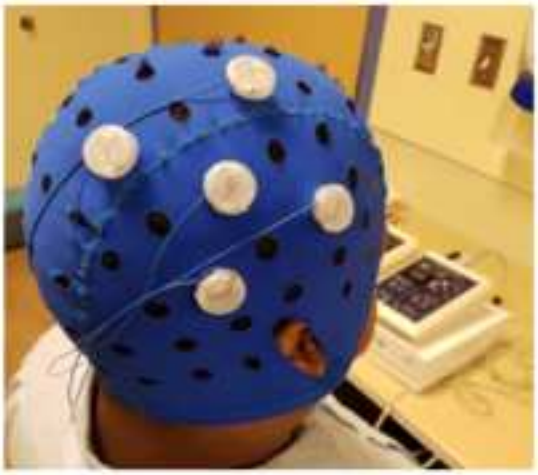

Figure 2

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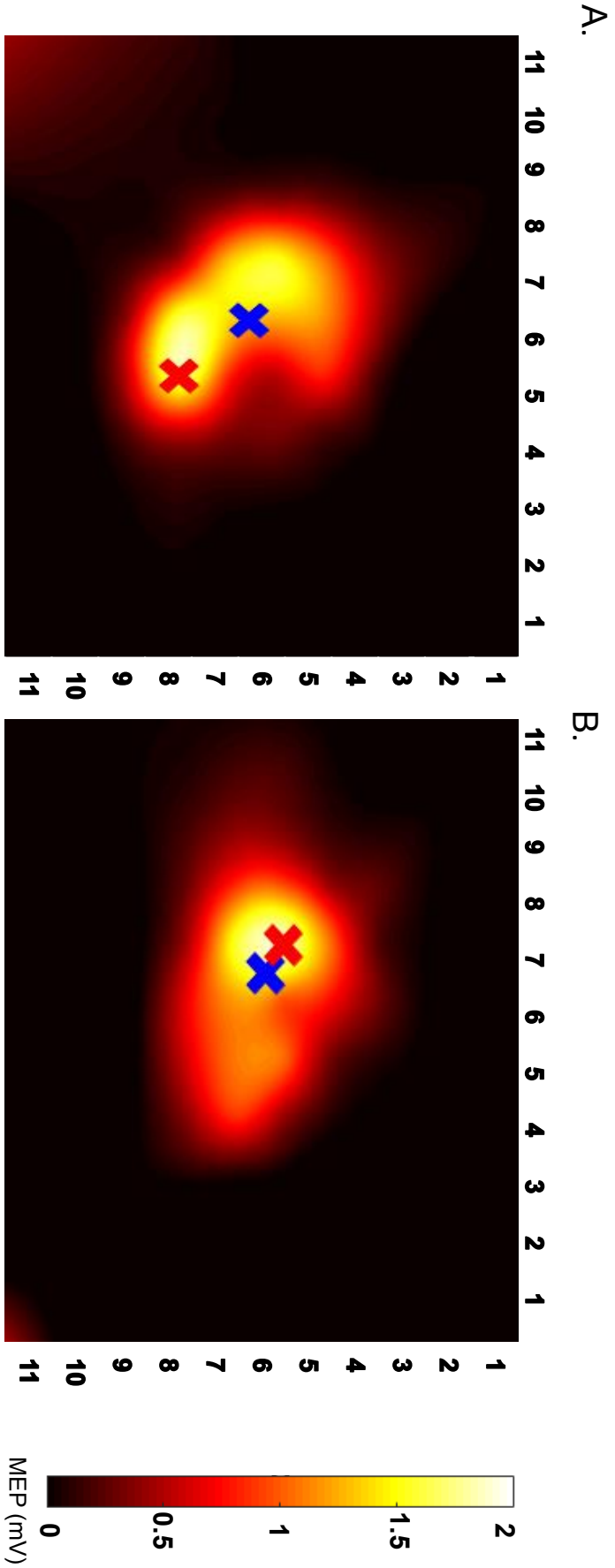

Figure 3

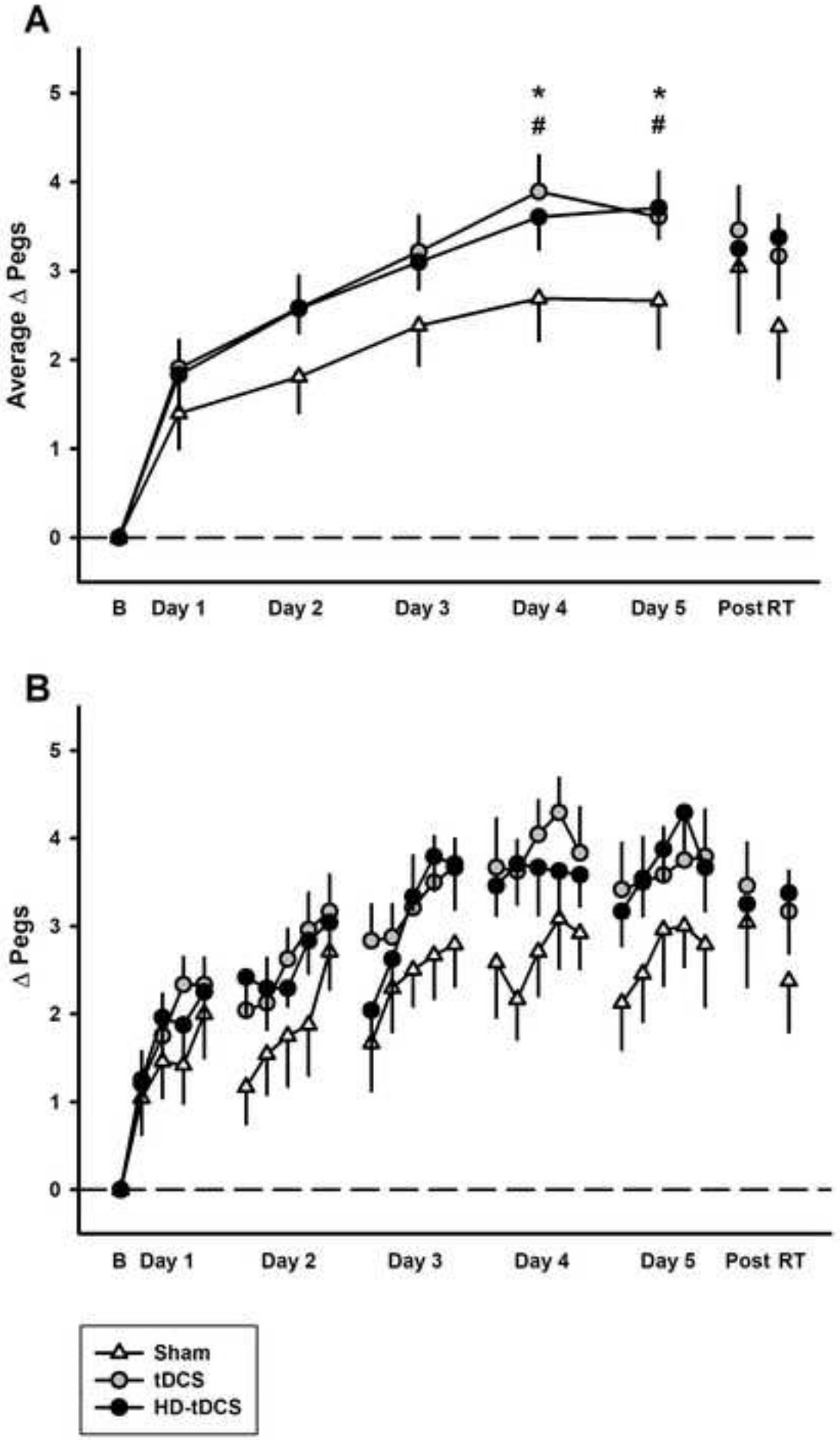

Figure 4

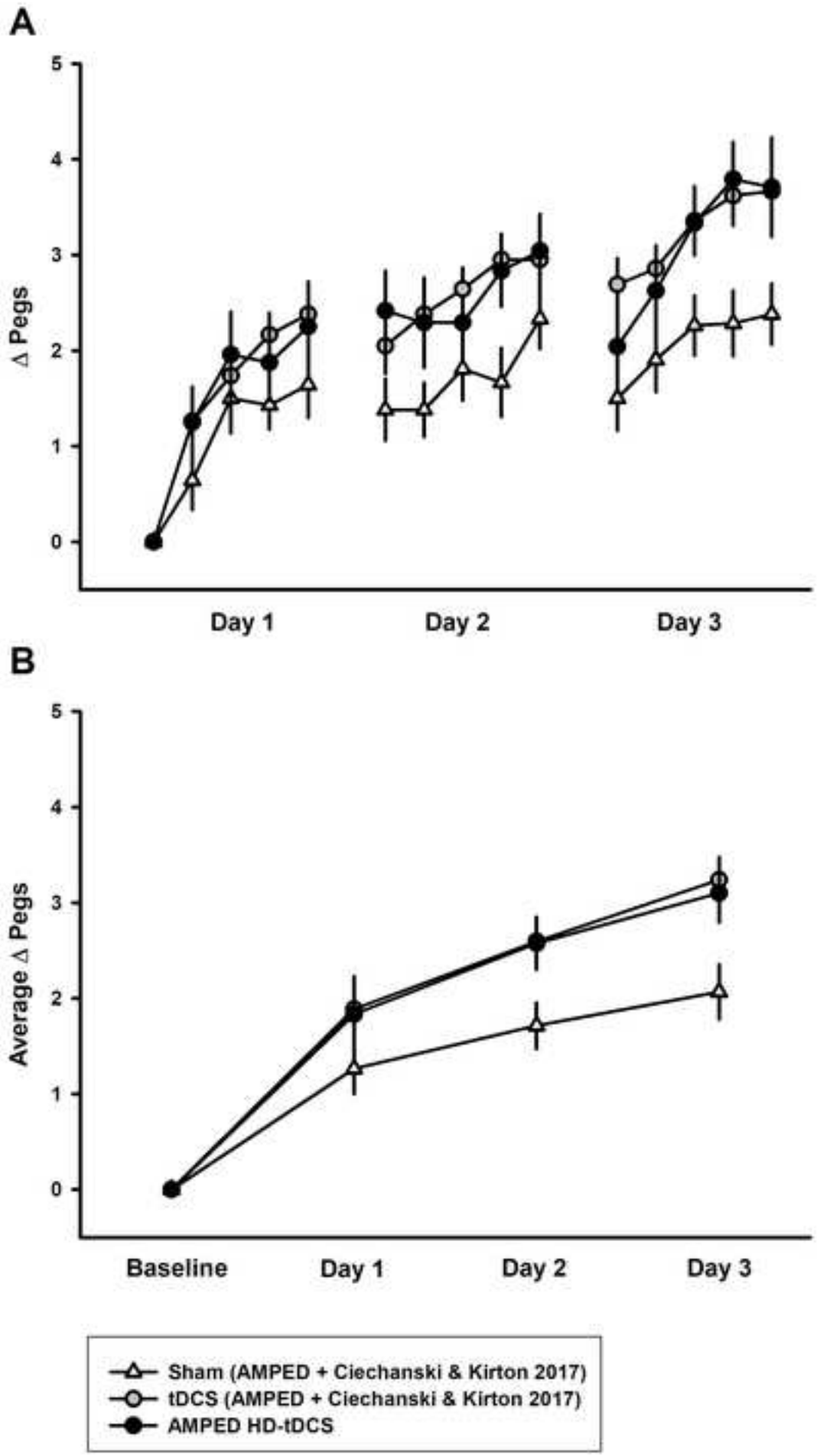

Table of Materials

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Name of Material/ Equipment	Company
1x1 SMARTscan Stimulator	Soterix Medical Inc.
4x1 HD-tDCS Adaptor	Soterix Medical Inc.
Brainsight Neuronavigation	Roge Resolution
Carbon Rubber Electrode	Soterix Medical Inc.
EASYpad Electrode	Soterix Medical Inc.
EASYstraps	Soterix Medical Inc.
EMG Amplifier	Bortec Biomedical
HD1 Electrode Holder	Soterix Medical Inc.
HD-Electrode	Soterix Medical Inc.
HD-Gel	Soterix Medical Inc.
Micro 1401 Data Acquisition System	Cambridge Electronic
Purdue Pegboard	Lafayette Instrument
Saline solution	Baxter
Soterix Medical HD-Cap	Soterix Medical Inc.
TMS Robot	Axilium Robotics
TMS Stimulator and Coil	Magstim Inc

Catalog Number	Comments/Description
https://soterixmedical.com/research/1x1/tdcs/device	
https://soterixmedical.com/research/hd-tdcs/4x1	
https://www.rogue-resolutions.com/catalogue/neuro-navigation/brainsight-tms-navigation/	
https://soterixmedical.com/research/1x1/accessories/carbon-ruber-electrode	
https://soterixmedical.com/research/1x1/accessories/1x1-easypad	
https://soterixmedical.com/research/1x1/accessories/1x1-easystrap	
http://www.bortec.ca/pages/amt_16.htm	
https://soterixmedical.com/research/hd-tdcs/accessories/hd	Standard Base HD-Electrode Holder for H
https://soterixmedical.com/research/hd-tdcs/accessories/hd	Sintered ring HD-Electrode.
https://soterixmedical.com/research/hd-tdcs/accessories/hd	HD-GEL for High Definition tES (HD-tES)
s http://ced.co.uk/products/mic3in	
Company	
http://www.baxter.ca/en/products-expertise/iv-solutions-premixed-drugs/products/iv-solutions.page	
https://soterixmedical.com/research/hd-tdcs/accessories/hd-cap	
http://www.axilumrobotics.com/en/	
https://www.magstim.com/neuromodulation/	

ligh Definition tES (HD-tES)

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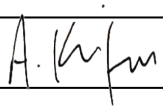
Institution:	University of Calgary
Title:	Professor
Signature:	
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Editorial comments:
The manuscript has been modified and the updated manuscript, ***59594_R0.docx****, is attached and located in your Editorial Manager account.* ***Please use the updated version to make your revisions.***

We thank the editor and reviewers for their thoughtful questions and suggestions. The paper is much improved as a result. An itemized response to each is below and accompanied by the revised manuscript with changes tracked.

Editorial comments

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: We have proofread the entire manuscript and corrected any spelling or grammar issues.

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Response: We have removed all company names and commercial language from the protocol. The list of software is included in Table 1: Table of Materials and Reagents.

4. Please ensure that the numbering of the protocol steps is correct (sub-steps under step 2, line 113).

Response: The numbering of protocol steps have been corrected.

5. Please add a one-line space between each of your protocol steps.

Response: Thank you for your suggestion. In order to select 2.75/4 pages, the document was left without a one-line space between each protocol step. This step can be added on the final proof.

6. Figures: Please define all error bars in the Figure Legends.

Response: Thank you for identifying this important omission. All error bars and symbols are now defined in the figure legends.

7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). Any text that cannot be written in the imperative tense may be added as a "Note."

Response: All text in the protocol section has been updated and is now written in the imperative tense.

8. For steps that are done using software, a step-wise description of software usage must be included in the step. Please mention what button is clicked on in the software, or which menu items need to be selected to perform the step.

Response: The protocol has been updated to include a step-wise description of software usage.

9. Please split some long steps into more sub-steps so that each step contains 2-3 actions and is less than 4 lines.

Response: Thank you for this suggestion. Nearly all steps and sub-steps have been changed to be less than 4 lines.

10. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol steps (including headings and spacing) in yellow that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Response: We have highlighted 2.75 pages for filmable content.

11. Please use h, min, s for time units.
Response: All units have been changed according to this comment.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:
The authors describe a method to advance the application of non-invasive brain stimulation in children. The authors describe a protocol for robotic transcranial magnetic stimulation (TMS) motor mapping. Individualized, MRI-navigated 12x12 grids centered on the motor cortex guide a robot to administer single-pulse TMS. Mean motor evoked potential (MEP) amplitudes per grid point are used to generate 3D motor maps of individual hand muscles with outcomes including map, area, volume, and center of gravity.

Major Concerns:
No major concern

Minor Concerns:
No minor concern. The protocol and its description are convincing and of a certain interest to the pertinent researcher community

Reviewer #2:

The authors report their protocol for the noninvasive mapping (robotic TMS) and modulation (HD-tDCS and tDCS) of the motor cortex in children. Thereby, they propose a way to standardize these procedures, while supporting the efficacy of the modulation protocol by a recently published study on modulation of motor skill learning. Since noninvasive stimulation parameters for children are less well established compared to adults, this protocol it highly relevant. The report is further easy to follow. In the following I will discuss my main comments on the protocol.

Major concerns:
1. While the paper proposes a protocol specifically for children, the report focusses very little on the specific issues and challenges related to measuring children.

Response: Thank you for your comment. We agree that the original protocol can be used in adults as well. In the revised version, we made less emphasis on children. Any specific consideration related to pediatric application are stated under ‘Notes’ section of each step.

Often children tend to move more compared to adults, it could be harder to assure muscle relaxation for the TMS procedure and their patience for the whole experiment might be limited. These points should be addressed and mentioned in the protocol, if such issues were experienced.

Response: Thank you for highlighting this important and practical issue. A note has been added to protocol step 2.3.2 regarding muscle relaxation. That individual tracings are reviewed for baseline muscle activity and excluded accordingly is also noted in the protocol.

This also includes the duration of breaks mentioned in Figure 1. Was this duration the same for all children, did it differ according to individual needs, were sometimes longer or more breaks necessary?

Response: All breaks had fixed duration for all participants. However, the motor mapping session duration varied depends on the number of responsive sites. As a result, the duration of the entire session varied between participants. Flexibility was also provided for specific circumstances for individual children within reason (i.e. had to go to bathroom). We do not have room to add these details to the protocol though believe they fall within common sense and can be adjusted accordingly for individual experimenters.

In this context, it would be also be helpful to have some estimations for how long each major step in the protocol takes on average.

Response: We agree this is an important consideration. An estimation for how long each major step in the protocol has been added.

Further, the authors do not state the age range that this protocol has been tested on or they propose it appropriate for. This information should be added.

Response: Thank you for highlighting this omission on our part. The age range has been defined in the results section.

2. Despite emphasizing the potential advantage of robotic TMS, this point is rarely discussed or mentioned in the protocol. It should be clearer, why the authors consider robotic TMS an advantage (especially also for mapping of children) and how this idea is supported. In the protocol it should be made clearer, which steps are taken over by the robot and which steps still must be done manually.

Response: We have attempted to better clarify the specific advantages of robotic TMS motor mapping. All steps involving TMS were done using the TMS Robot with each specific step documented in the protocol. Many steps would be possible to complete manually but less consistency and accuracy in coil placement would be expected. These and the other potential advantages of the TMS robot are now reviewed in the discussion. As out study was not a direct comparison between manual and robotic methods, we have avoided further speculation.

3. The advantage of HD-tDCS for replication and future studies is not clear from the results

stated. In their previous study the authors show that conventional tDCS and HD-tDCS have similar effects on motor learning. However, in the following (e.g. line 80-82, line 347-350) parts HD-tDCS is presented as potentially more promising technique. This conclusion is not supported by the stated results and needs further references or elaboration (e.g. why the authors expect HD-tDCS to increase replication).

Response: Thank you for these comments. Our study here is intended to describe the methodology of applying both tDCS and HD-tDCS in children as this has not been described before. We are not intending or able to demonstrate any particular advantages or disadvantages. As discussed by Alam et al. 2016, HD-tDCS likely produces more focal targeting which may increase replication of the effect compared to conventional tDCS. Statement and reference have been added to the discussion section.

Minor concerns:
4. According to the JoVe Guidelines it is necessary to mark the parts of the protocol that are supposed to be illustrated in the video, if this protocol exceeds 3 pages. Thus, please mark max. 2.75/ 4 pages.

Response: The protocol has been marked for the relevant 2.75 pages.

5. P.3/ l. 119-124: Please clarify, how the neuronavigation is implemented in the protocol. It is unclear, if only the registration or also TMS stimulation itself are performed neuronavigated? Why was a marker used to mark the hotspot and not neuronavigation? Please also add some practical comments on the following question: Was the marker still visible on the following days, since this step was not repeated on the following days? Usually marker points fade away fast especially if saline soaked electrodes are placed on top.

Response: Thank you for your comment. Line 3.5 has been changed to better describe the neuronavigation elements of the protocol.

6. P.4 / l. 163- 164: Please clarify, what is regarded as optimal/ sub-optimal contact quality?

Response: We have added further clarification regarding contact quality in step 3.5.1.

7. P. 5/ l. 190: Here it sounds, like something is happening in the sham group after 20 minutes. Please clarify this point and add a brief note on the rationale behind ramping down again after 20 minutes (if this was the case).

Response: Such protocols for Sham groups are quite standardized across the tDCS field. In fact, having the current ramp up to 1mA and back down to 0mA in the first minute and final minute of stimulation is how the tDCS device is set up to function. For >90% of the protocol, the Sham group receives 0mA of stimulation.

8. In the protocol, points 2.3 & 2.4. are phrased in a confusing way. Please clarify the

procedure. For point 2.3: 4 pulses at each intensity step starting at 100% MSO are applied here? Why was this protocol chosen (e.g. standard would be 5 out of 10 pulses for RMT determination)? Why is a high stimulation intensity of 100% MSO used/ needed? For point 2.4: For each grid, 4 stimulations are applied. If > 50% of these stimulations are positive, stimulation proceeds to the next grid. A non-responsive point would correspond to < 50% positive stimulations in one grid?

Response: Thank you for identifying the need for better clarity in this section of the methods. The number of stimulations noted was for mapping, not setting of RMT. For efficiency, RMT was calculated by producing stimulus recruitment curve (SRC), starting from 100% MSO as most children's MEP plateau at this intensity. This step of determining RMT is now clearly described in the protocol (see 2.4.6).

9. P. 5/ l. 192: Please add a reference for the questionnaire or rephrase to clarify, if it is a custom-made questionnaire.

Response: The questionnaire reference has been added.

10. P. 5/ l. 228 - 231: Please also mention placement of the ground electrode.

Response: We have now indicated where the ground electrode is placed (2.3.3).

11. P.6/ l. 245: Is the MATLAB script available somewhere? Otherwise, please clarify the threshold determination procedure (e.g. by one example).

Response: The method of determining RMT has been changed as noted above and no longer requires the MATLAB script.

12. P. 6/ l. 273: Please add exact p-values for $p<0.044$ and $p<0.05$

Response: The exact values have been added.

13. Figure Legends:
*Figure 2: Please add legends for * and #. Please add number of subjects for each group.*
Figure 2/ 3: Please add labels for error bars.
Figure 4: The image quality is a bit low (i.e. the axes descriptions are difficult to read). Please add labels for red and blue cross and color scale for the image.

Response: The figure legends have been changed accordingly. Figure 4 has been re-created and uploaded.

14. Typos:
P.2/ l. 72: differences
P.2/ l. 78: towards

P.2/ l. 85: safe
P.2/ l. 92: maps
P.3/ l. 112: Numbering wrong
P.3/ l. 139: delete "into the cap" as it repeats
P.4/ l. 154: on top of the gel
P.5/ l. 190: delete "have"
P.6/ l. 232: with the EMG
P.6/ l. 245: Numbering wrong

Response: Thank you. All typos have been corrected.

Reviewer #3:

I would like to compliment the authors on a detailed outline of their procedures with respect to the application of tDCS and TMS mapping. Standardization of methods in the field of brain stimulation is important to allow better comparison of results and this methods manuscript is a step in the right direction.

I have two general remarks and various specific comments which may help the authors to improve their manuscript:

General points:

** Although I acknowledge the primary interest of the authors is in pediatrics - the described methods are not exclusive of specific for application in the developing brain. The described methods are pretty general - and apart from the robotic TMS - used across all study populations. This is not to say the methods should not be presented - but require either (1) a better presentation with respect to which procedures are tailored for use if paediatrics; or (2) less emphasis on the application in paediatrics starting with the title of the manuscript.*

Response: Thank you for your comment. We agree that our protocols can be directly applied to adults where tDCS methods are well established but robotic motor mapping is perhaps more novel. One of our aims was to promote the feasibility of such methods in young patients where non-invasive brain stimulation applications have been far more limited. This includes some unique pediatric issues for both tDCS (where HD studies have not yet been described) and some unique elements of motor mapping. Therefore, in the revised version, we placed less emphasis on children but maintained inclusion of pediatric specific elements under the 'Notes' section of each step.

** In line with the previous point it would be good for the authors to include a few more references, specifically linked to tDCS&learning (e.g. Woods et al., 2016 Clin Neurophysiol, Nitsche et al., 2003 J Cog Neurosci) and TMS mapping methodology (Julkunen et al. 2014, J Neurosci Meth, van de Ruit et al., 2015 Brain Stim) in introduction and discussion.*

Response: We agree and multiple references have been added.

Specific comments:

** Step 1.18: Can the authors include this questionnaire? Or cite to the study where it is presented?*

Response: The questionnaire reference has been added.

** Step 2.3.3: I don't understand this description. Surely, the 'minimum MEP' is close to 0 (background EMG) - so why not 5% of max? Where does the 5% come from? As this procedure seems non-conventional (neither the Rothwell/Rossini method nor a PEST algorithm) it should be better explained.*

Response: As addressed in the comment from reviewer 2 above, an SRC method was employed to set RMT with greater efficiency. The protocol has been updated accordingly (see 2.4.6).

** Step 2.3.3: Starting at 100% MSO for threshold hunting is excessive. Why would you do that to children? It will scare them and make them tense up, potentially affecting the rest of the TMS measures. I find this inappropriate, specifically for the use advocated here and suggest using a different threshold criteria where there is no need to do this.*

Response: We appreciate the reviewers concern and can reassure them that the protocol was developed very carefully with input from children themselves. Our lab has delivered over 3 million stimulations to >350 children with many different neurological disorders and very young ages. All subjects naïve to TMS are first introduced to the process and equipment and allowed to ask questions. They also receive gentle test stimulation to familiarize them with the sensation which are then gradually elevated to ensure tolerance. Only after this are 100% MSO stimulations delivered where tolerability has been excellent with no subjects asking to stop or withdraw despite being repeated offered the opportunity to do so. These important pediatric-specific details are now included in the protocol.

** Step 2.3.3 included twice.*

Response: This step was replaced by Step 2.4.6 using a method that can be more easily reproduced and that is common in the literature.

** Step 2.4.1: What was the interstimulus interval?*

Response: This ISI is now indicated as step 2.5.1

** Step 2.5.1: Include scripts or describe processing so others can reproduce.*

Response: A user-friendly version of the map creation MATLAB script is being developed to share with the public as opensource. Meanwhile, the corresponding author can be contacted to obtain free access to the script.

** Step 2.5.1: I guess information with respect to stimulation position was exported from BrainSight to create the maps? This should be included.*

Response: We used a hard copy version to track the stimulation order and used this information to create the maps. This is added as a step in the protocol (2.5.8).

** I feel it is important to mention somewhere the TMS robot is not a requirement to perform the mapping procedure. While it is convenient, and may improve consistency of the TMS mapping results for unexperienced TMS users, it is not a necessity to perform the TMS mapping procedure. As many readers will not have this robot - this may put them off in adopting the methods.*

Response: We agree though one intention of this procedural protocol was to describe the robot method specifically. That a parallel approach could be employed using handheld TMS is now noted in the discussion.

** Representative Results: Please include some results on how many stimuli / grid points where required for motor mapping to get an indication of how long the procedure took.*

Response: The average number of grid-points per map is 35-40. The procedure took on average 18 minutes per hemisphere, and on average 36 minutes for bilateral mapping. These important, practical details are now mentioned in step 2.5.6.

** TMSMM (line 312) is not defined in the text (only the figure caption).*

Response: The text and figure caption have been updated.

** Ref 16 (line 362) does not include any TMS motor mapping.*

Response: Thank you for noticing this error on our part. The reference has been corrected.

** Discussion on TMS mapping should include something on acquisition time which is important for application in paediatrics and other vulnerable populations and has been an active area of research.*

Response: We agree and this has now been addressed as noted above.

** Figure 4: Missing units for colorbar. No indication of anterior/posterior or medial/lateral. Which cross is hotspot, which one the COG?*

Response: The figure legends now indicate the position and which crosses represent the hotspot and COG.

Thursday, April 11, 2019

Editorial comments:
The manuscript has been modified and the updated manuscript, ***59594_R2.docx****, is attached and located in your Editorial Manager account.* ***Please use the updated version to make your revisions.***

We thank the editor and reviewers for their thoughtful questions and suggestions. All additional editorial suggestions have been incorporated. Please let us know if there are any additional changes needed.