Video Article

The Use of Magnetic Resonance Spectroscopy as a Tool for the Measurement of Bi-hemispheric Transcranial Electric Stimulation Effects on Primary Motor Cortex Metabolism

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Abstract

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that has been increasingly used over the past decade in the treatment of neurological and psychiatric disorders such as stroke and depression. Yet, the mechanisms underlying its ability to modulate brain excitability to improve clinical symptoms remains poorly understood [33]. To help improve this understanding, proton magnetic resonance spectroscopy (^{1}H-MRS) can be used as it allows the *in vivo* quantification of brain metabolites such as γ-aminobutyric acid (GABA) and glutamate in a region-specific manner [41]. In fact, a recent study demonstrated that ^{1}H-MRS is indeed a powerful means to better understand the effects of tDCS on neurotransmitter concentration [34]. This article aims to describe the complete protocol for combining tDCS (NeuroConn MR compatible stimulator) with ^{1}H-MRS at 3 T using a MEGA-PRESS sequence. We will describe the impact of a protocol that has shown great promise for the treatment of motor dysfunctions after stroke, which consists of bilateral stimulation of primary motor cortices [27,30,31]. Methodological factors to consider and possible modifications to the protocol are also discussed.

Video Link

The video component of this article can be found at https://www.jove.com/video/51631/

Introduction

The idea of applying electricity to the human brain to modulate its activity has been studied since ancient times. In fact, writings from as early as the 11th century have been found that describe the use of the torpedo electric fish in the treatment of epileptic seizures[1]. Yet, it is not until recently that non-invasive brain stimulation has received widespread interest in the scientific community as it was shown to produce modulatory effects on cognitive function and motor response[2]. While transcranial magnetic stimulation (TMS) has been extensively studied since the early 1980's[3], recent interest in transcranial direct current stimulation (tDCS) has increased as it is now considered a viable treatment option for a wide range of neuropathologies, such as stroke[4], alcohol addiction[5], and chronic pain[6]. tDCS has many advantages over neurostimulation techniques like TMS, for example, since it is relatively inexpensive, painless, well tolerated by patients, and portable, thus making it possible to administer at bedside[7]. In fact, only a small percentage of patients experience a mild tingling sensation during stimulation[8]. However, this sensation usually disappears after a few seconds[9]. Consequently, tDCS allows robust double-blind, sham-controlled studies since a majority of participants cannot differentiate sham stimulation from real stimulation[9,10].

tDCS involves the induction of a constant low-amperage electric current (1-2 mA) applied to the cortex via surface electrodes positioned on the scalp of the subject. The electrodes are usually placed into saline-soaked sponges or directly on the scalp with an EEG-type paste. To conduct a tDCS study, four main parameters need to be controlled by the experimenter: 1) the duration of stimulation; 2) the intensity of stimulation; 3) the electrode size; and 4) the electrode montage. In standard protocols, the “active” electrode is positioned over the region of interest while the reference electrode is usually placed over the supraorbital region. The current flows from the positively charged anode towards the negatively charged cathode. The effect of tDCS on primary motor cortex (M1) is determined by the polarity of the stimulation where anodal stimulation enhances the excitability of a population of neurons and cathodal stimulation reduces it [11]. Unlike TMS, the induced current is insufficient to produce action potentials in cortical neurons. The changes in cortical excitability are believed to be due to the modulation of the membrane neuronal threshold leading to either the hyperpolarization of membrane potentials or a facilitation of depolarization of neurons depending on the direction of the current flow [8,11]. The duration of the excitability changes can persist for up to 90 min after the offset of stimulation, depending on stimulation duration [11,12].

tDCS and Motor Rehabilitation

The M1 has been extensively used as a target of stimulation since excitability changes elicited by tDCS can be quantified through motor evoked potentials (MEPs) induced by single pulse TMS [3]. Early studies showing the possibility of measuring polarity-specific excitability changes induced by tDCS have used M1 as a target of stimulation [11,12]. Since then, M1 has remained one of the primary targets of tDCS in studies involving both clinical populations and healthy subjects because of its importance in motor function, memory formation, and consolidation of motor skills [12].

The brain relies on a complex interaction between motor regions of both hemispheres to perform a movement [14]. When one area is damaged, after suffering a stroke for example, inter-hemispheric interactions are altered. Studies on brain plasticity have shown that the motor areas of the brain adapt to this modification in different ways [15]. First, the intact, surrounding regions of the damaged area can become overactived, leading to inhibition of the damaged area - a process called intra-hemispheric inhibition. Second, the homologous region of the damaged area can become overactivated and exert inhibition on the injured hemisphere - a process called inter-hemispheric inhibition. The affected M1 can therefore be twice penalized: first by the lesion and second by the inhibition coming from both the unaffected M1 and the surrounding region of the affected M1 [16]. A recent study has shown that increased excitability in the unaffected hemisphere is linked to slower rehabilitation [17], which has been described as maladaptive inter-hemispheric competition [18].

Understanding the plasticity occurring after a stroke may lead to the development of neuromodulation protocols that can restore interhemispheric interactions [19]. Three main tDCS treatments have been proposed in patients with motor deficits following stroke [20,21]. The first treatment aims to reactivate the injured motor cortex by unilateral *anodal* stimulation (a-tDCS). In this case, stimulation aims at *directly* increasing activity in perilesional areas, which are believed to be essential for recovery. In fact, studies have shown improvement of the paretic upper or lower limb following this treatment [22-26]. The second treatment was developed with the aim of reducing the over-activation of the contralesional hemisphere by applying unilateral *cathodal* tDCS (c-tDCS) over the intact M1. Here, stimulation aims at *indirectly* increasing activity in perilesional areas through interhemispehric interactions. Results from these studies have shown improvement of motor function after c-tDCS [4,27-29]. Finally, the third treatment aims at combining the excitatory effects of a-tDCS over the injured M1 with the inhibitory effects of c-tDCS over the unaffected M1 using *bilateral* tDCS. Results have shown improvements in motor function after bilateral tDCS [27,30,31]. Moreover, one study demonstrated greater improvements following bilateral tDCS compared to both unilateral methods [32].

Physiological Mechanisms of tDCS

Despite the increasing use of tDCS in the treatment of stroke, the physiological mechanism underlying its effects remains unknown [33]. A better understanding of the physiological effects could help develop better treatment options and could lead to standardized protocols. As mentioned earlier, the effects of tDCS can last for up to 90 min after the offset of stimulation [11,12]. Therefore, hyperpolarization/depolarization processes cannot completely explain long lasting effects [33,34]. Different hypotheses have been suggested regarding the physiological mechanism underlying tDCS after-effects on M1 including changes in neurotransmitter release, protein synthesis, ion channel function, or receptor activity [34,35]. Insights into this matter were first acquired through pharmacological studies showing a suppression of the after effects of anodal and cathodal stimulation on M1 excitability by the glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist dextromethorphan[36,37] whereas the opposite effect was shown using a NMDA receptor agonist[38]. NMDA receptors are thought to be involved in learning and memory function through long term potentiation (LTP) and long term depression (LTD), both mediated by glutamatergic and GABAergic neurons[39,40]. Animal studies are in line with this hypothesis as they have shown that a-tDCS induces LTP[13].

Despite the important progress made in our understanding of the mechanisms of action underlying tDCS effects, pharmacological protocols present important limitations. Indeed, drug action cannot be as spatially specific as tDCS, especially in the context of human experimentation, and the mechanism of action of their effects is mostly due to post-synaptic receptors [34]. Therefore, there is a need to investigate more directly the effects of tDCS on the human brain. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a good candidate as it allows non-invasive *in vivo* detection of neurotransmitter concentrations in a specific region of interest. This method is based on the principle that every proton-containing neurochemical in the brain has a specific molecular structure and consequently, produces chemically specific resonances that can be detected by ^{1}H-MRS [41]. The acquired signal from the brain's volume of interest is generated from all protons that resonate between 1 and 5 ppm. The acquired neurochemicals are represented on a spectrum and plotted as a function of their chemical shift with some clearly distinguishable peaks, but where many resonances from the different neurochemicals overlap. The signal intensity of each peak is proportional to the concentration of the neurometabolite [41]. The amount of neurochemicals that can be quantified depends on the strength of the magnetic field [42,43]. However, low-concentration metabolites, which are obscured by very strong resonances, are hard to quantify at lower field strength such as 3 T. One way to obtain information about such overlapping signals is to remove the strong resonances via spectral editing. One of such techniques is a MEGA-PRESS sequence, which allows detection of γ-aminobutyric acid (GABA) signals [44,45].

Only a few studies have investigated the effect of tDCS on the brain metabolism using ^{1}H-MRS in motor [34,46] and non-motor regions[47.] Stagg and collaborators [34] assessed the effects of a-tDCS, c-tDCS, and sham stimulation on M1 metabolism. They found a significant reduction in GABA concentration following a-tDCS, and a significant reduction of glutamate+glutamine (Glx) and GABA following c-tDCS. In another study, it was reported that the amount of changes in GABA concentration induced by a-tDCS over M1 was related to motor learning [46].

These studies highlight the potential of combining ^{1}H-MRS with tDCS to increase our understanding of the physiological mechanism underlying the effect of tDCS on motor function. In addition, the use of clinical protocols such as a-tDCS and c-tDCS over M1 is useful because their behavioral effects are well studied and can be directly related to physiological results. Therefore, a standard protocol for combining bilateral tDCS and ^{1}H-MRS is demonstrated in healthy participants using a 3 T MRI system. Bihemispheric tDCS is presented to contrast data with a previous MRS study where unilateral cathodal or unilateral anodal tDCS were applied over motor cortex [34]. The protocol is described specifically for stimulation with a NeuroConn stimulator in a Siemens 3 T scanner performing MEGA-PRESS ^{1}H-MRS.

Protocol

The study was approved by the Research and Community Ethics Boards of Unité de Neuroimagerie Fonctionnelle and University of Montréal and was done in compliance with the code of ethics as stated in the Declaration of Helsinki. All subjects gave written informed consent following careful screening for MRI compatibility and were financially compensated for their participation.

1. tDCS Material

1. Make sure all necessary materials are available before starting the experiment (see **Figure 1** for list).
   Note: Different electrode sizes are available for tDCS. For this study, two 5 x 7 cm rubber electrodes will be used. Other sizes can be chosen depending on the area of stimulation and the desired focality of the stimulation [48].
2. Make sure to check that the batteries of the DC-stimulator are charged and to periodically charge them since the device cannot be charged or plugged-in during stimulation for safety reasons.

2. Planning of the Conditions for Stimulation

1. Turn on the tDCS device according to the instructions included with the device. Pre-set the tDCS device for two different stimulation modes (active and sham).
2. As some devices do not have a pre-set mode, select the appropriate sham parameters before starting stimulation.
   1. Pre-define a set of parameters by loading a setting. Press the button 2 or 4 to select from the main menu the "system" option (see **Figure 2**).
   2. Move the cursor to line 2 on the display by pressing the button 3.
   3. Press the button 2 or 4 until the "load setting" shows on the display. Press the button 3.
   4. Select the letter of the setting (A, B, C or D) by pressing the button 2 or 4.
   5. Move the cursor upwards with the button 1. The display will automatically show the "parameters" option.
   6. Set the tDCS device to a current of 1 mA. To do so, press the button 1 to select the line 3 of the "parameters" menu of the display. Select the "current" option by pressing the button 2 or 4. Press the button 3 to reach the line 4 and modify the intensity to 1,000 µA by pressing the button 2 or 4.
   7. Press the button 1 to go back to the line 3. Select the "fade in" option from the screen menu of the device by pressing the button 2 or 4. Press the button 3 to go to the line 4 and press the buttons 2 and 4 to adjust the duration to 15 s.
      Note: Fade in durations can be modified.
   8. Press the button 1 to go back to the line 3. Select the "fade out" option from the screen menu of the device by pressing the buttons 2 or 4. Press the button 3 to go to the line 4 and press the buttons 2 and 4 to adjust the duration to 15 s.
      Note: Fade in durations can be modified.
   9. Press the button 1 to go back to the line 3. Press the button 2 or 4 until the "duration" option shows on the display menu. Press the button 3 to go to the line 4 and press the button 2 and 4 to adjust the duration to the minimum duration available on the device (15 s for the present device; see **Figure 3b**).
      Note: This will induce a tingling sensation similar to the active stimulation.
3. Press the buttons 1 and 3 simultaneously to save the changes of the setting.
4. Pre-program the active stimulation parameters. To do so, follow the same instructions as for the setting of the sham stimulation, but program the duration to 1,200 sec (20 min; see **Figure 3a**).
5. Pre-program the test stimulation parameters. To do so, follow the same instructions as for the setting of the sham stimulation but program the duration to 45 sec.
   Note: The test stimulation will be used for the measurement of the impedance prior to the experimentation.
6. Pseudo-randomly assign the conditions of stimulation to participants.
7. Assign a number to each of the three conditions for a blind experimentation: 1) bilateral: anodal right, cathodal left; 2) bilateral: anodal left, cathodal right; 3) sham: anodal right, cathodal left.

3. Consenting the Participants

1. Inform the participant of the procedure and sign consent form.
   1. Verify that participants do not have any contraindication to tDCS: a psychiatric or neurological history, the presence of a pacemaker, metal implanted in skull, a history of fainting, a history of seizures, a history of substance abuse, a family history of seizure, a history of febrile fits, a lack of sleep in the preceding night, a history of skin sensitivity, and any alcohol consumption the previous day.
   2. Inform the participant of the most reported side-effects of tDCS: mild tingling; moderate fatigue; light sensation of itching under the electrodes; slight burning sensation.
2. Inform the participant of the usual MR contraindications and side effects.

4. Measurements for Electrodes Placement

1. Use the 10/20 international system to find the following landmarks on the participant head: nasion and inion (**Figure 4a**), preauricular points, and the two targeted areas: C3 and C4 (**Figure 4b**).

1. Locate the nasion as the distinct depressed area located on the bridge of the nose at the level between both eyes. Locate the inion as the most prominent projection of the occipital bone located at the lower part of the skull. Locate the preauricular point near each ear; it is the indentation above the zygomatic notch. Locate the C3 and C4 based on measurements as described below.

2. Use a measuring tape to measure the distance between the nasion and inion along the midline of the head and make a mark at 50% of the distance with a non-permanent hydro marker.
3. Use a measuring tape to measure the distance between the two preauricular points and make a mark with a non-permanent hydro marker at 50% of the distance in line with the previous mark. This point corresponds to Cz (vertex).
4. From the Cz, along the line created between the preauricular points, mark two points, one on each side, with a non-permanent hydro marker that correspond to 20% of the total distance. These marks correspond to the target areas (C3 and C4, **Figure 4b**).
   Note: Other methods such as TMS or neuronavigation can also be used to localize M1.

5. Placement of Electrodes

1. Move as much hair as possible away from the targeted areas that will be stimulated. Apply an EEG-type exfoliating gel with a cotton-swab to clean the targeted areas.
2. Clean the targeted areas with a 70% isopropyl alcohol and pumice prepping pad to enhance electrode contact.
3. Generously cover the entire electrode with an EEG-type conductive paste. Ensure that the paste is approximately 5 mm thick across the entire surface. Make sure the entire rubber area is covered with paste. Lightly wet the target areas and the conductive paste on the electrodes with a saline solution.
4. Position the electrodes as shown in **Figure 4b** and press the electrodes firmly onto the targeted areas. Place a rubber band around the head of the participant to ensure optimal stability of the electrodes. Adjust it in such a way that the participant will experience no pain or discomfort during the scanning session.
5. Make sure that the leads do not come in contact with the skin to avoid potential burns.

6. tDCS Test Outside the Scanner Room

1. Use a multimeter to verify the proper functioning of the electrode cable and resistance.
2. Turn on the tDCS device and load the test stimulation settings.
   1. Press the button 2 or 4 to select from the main menu the "system" option. Move the cursor to line 2 on the display by pressing the button 3. Press the button 2 or 4 until the "load setting" shows on the display. Press the button 3. Select the letter of the pre-programmed test setting (A, B, C or D) by pressing the button 2 or 4.
   2. Move the cursor upwards with the button 1. The display will automatically show "parameters" option. On the first line, press the button 2. The display will show "stimulation?" with the different pre-programmed parameters.

3. Press the button 1 to start the stimulation. The display will show the impedance level and automatically stop if it reaches more than 20 kΩ. If the impedance level is over 20 kΩ, unplug the electrode wires from the inner box and exit the scanning room to verify the positioning of the electrodes.
4. Redo the test stimulation. When a good level of impedance is reached and when the test stimulation is over, unplug the electrodes from the inner box.

7. tDCS Setup

1. As shown in **Figure 5**, place the tDCS device and the outer box in the scanner control room.
   Note: The tDCS device and the outer box are not MR compatible and should not be taken into the magnet environment.
2. Plug the outer box wires into the tDCS device and then plug the long box cable into the outer box.
3. Run the tDCS box cable from the scanner control room into the MRI room. Make sure to run this cable as straight as possible, avoiding any kinks or loops, along the wall of the MRI room towards the back of the MRI scanner. Put multiple MR compatible sandbags on the cable to ensure its stability, as shown in **Figure 5**.
4. Bring the inner box into the MRI room and plug the long box cable into it (**Figure 5**).

8. MRI Scan Preparation

1. Ask the participant to enter the MRI room, if not already in there from the tDCS test, and to put in earplugs.
2. Put a thin cushion under the coil area of the MRI table. Ask the participant to lie down on the table. Put a cushion under the legs of the participant for comfort and a blanket if needed. Give the participant the alarm button for security purposes.
3. Put separate headphones over both ears to allow transmission of information from the scanner control room to the participant in the MRI room.
4. Position the participant's head as high as possible under the area where the head coil will be positioned (top of the head as close as possible to the top of the table where the coil will be placed). Put the electrode wires along the right side of the head of the participant, as recommended by the tDCS device company.
5. Place the 32-channel receive-only coil around the head of the participant. Run the electrode cables through the right side of the coil. Position the head of the participant as straight as possible using a red positioning laser (built-in feature of the scanner).
6. Ask the participant to move arms and legs into a comfortable position, while making sure that hands do not touch. Make sure to remind the participant to stay as still as possible during the entire session. When the participant is ready, move the table past the middle line to reach the electrode wires at the back of the scanner.
7. Use medical tape to stabilize the electrode cable on the right side of the back of the coil. Plug the electrode wires located inside the scanner into the tDCS inner box. Put the inner box on the right side of the scanner with a sandbag on it for maximal stability.

8. Move the table back into its final position. Keep the tDCS turned on and the electrodes plugged into the outer box for the entire MRI session.

9. Pre-tDCS ^{1}H-MRS Session

1. Run a localizer sequence to acquire images needed to verify the proper positioning of the head and to compare to a second localizer which will be acquired at the end of the session to check for overall movement.
2. Acquire anatomical T_1-weighted MPRAGE images for the positioning of the M1 voxel and detection of possible structural abnormalities (T_R = 2,300 msec; T_E = 2.91 msec; FA: 9°; FOV = 256 x 256 mm; 256 x 256 matrix; T_I : 900 msec; 176 slices; orientation: sagittal; acquisition time: 4 min 12 sec).
3. Perform a multi-planner reconstruction of the images in planes that are more appropriate for visualization of the spectroscopy volume-of-interest (VOI).
    1. In the 3D card, browse the MPRAGE raw images (sagittal orientation). From the "creating parallel ranges" window select "axial 2X2". Adjust the position of the parallel lines and click on save to create the axial orthogonal view.
    2. From the "creating parallel ranges" window select "coronal 2X2". Adjust the position of the parallel lines and click on "save" to create the coronal orthogonal view.
4. Locate the left M1 based on Yousry and collaborators' [49] anatomical landmarks on the three orientation slices. Then, position the VOI (30 x 30 x 30 mm^3) on the area without any angulation relative to the scanner axis (figure 6).
5. Acquire a line-width scan (21 s).
    1. Select the spectroscopy card to measure water line-width on the real part of the signal from this line-width scan. Load the line-width raw data from the browser. Load the line-width measurement protocol (protocols menu: select the protocol).
    2. Adjust the phase using the scanner software interactive post-processing tools. Select the phase correction section and adjust the phase for the baseline with the cursor.
    3. In order to reduce the line-width, run the FAST(EST)MAP [50] sequence three times. Repeat the line-width scan and the line-width measurement (step 9.5). Note the final water line-width.
6. Start 4 blocks of 64 metabolite scans (32 "EDIT OFF" and 32 "EDIT ON", interleaved) with a MEGA-PRESS sequence [44,45], where VAPOR [51], OVS [51] and individual storage of FIDs are enabled (T_R = 3 s, T_E = 68 msec, total acquisition time: 12 min)
7. Acquire a water reference using MEGA-PRESS sequence without MEGA water suppression, with VAPOR suppression ("only RF off") and with a delta measurement at 0 ppm. Acquire a single block of 4 metabolite scans instead of 64 (acquisition time: 42 sec).

10. tDCS Procedure

1. Inform the participant that the tDCS stimulation will start and that the scanner will be silent for the entire stimulation.
2. Select one of the two previously programmed parameters according to the condition and start the stimulation. Keep track of the impedance and voltage during the 20 min of stimulation. When the stimulation is over, notify the participant that the post-tDCS MRS session will begin. Do not turn off the tDCS device.

11. Post-tDCS ^{1}H-MRS Session

1. Run the same metabolite scans with MEGA-PRESS sequence as the pre-tDCS scan but double the blocks of acquisition (8 blocks of 64 scans (32 "EDIT OFF" and 32 "EDIT ON", interleaved)) to acquire the metabolites at two different time points post-tDCS.
2. As with the pre-tDCS session, acquire a water reference scan using the same parameters. Finish the session with a localizer sequence.
3. Visually compare the localizer images acquired at the beginning and end of the scanning session as an index of head motion.
4. Access the viewing card and go to the browser menu. Select the first and second localizer raw images. Load the images in the viewing card and compare both images. Export data in the dicom format through the server.

12. Analysis of the ^{1}H-MRS Data

1. Import data using a programming and processing software, and adjust frequency and phase of individually stored FIDs using tCr and tCho signal between 2.85 and 3.40 ppm. To do so, use the software's lsqnonlin function to fit frequency and phase of each individual Fourier-transformed FIDs (spectra) to the average spectra of the session.
   Note: this is a site-specific approach and other methods for importing and analyzing data will not necessarily affect data quality.
2. To obtain the final spectra, subtract the signals from alternate scans with the selective double-banded pulses that were applied at 4.7 ppm and 7.5 ppm ("EDIT OFF") and at 1.9 ppm and 4.7 ppm ("EDIT ON") (**Figure 7**).
3. Use the LCModel [52] for the analysis of both difference and "EDIT OFF" spectra. Deactivate default simulations and baseline modeling.
4. Perform a visual inspection of the spectra to exclude sessions with contamination from subscapular lipid signal (see F**igure 9**).
5. As part of quality control, exclude spectra with linewidth of tCr-CH_3 above 10 Hz. Only include in the analysis metabolites (GABA, Glx, tCr, tNAA) which were quantified with Cramer-Rao lower bounds (CRLB) lower than 35%.
   Note: CRLB provide estimated error of the metabolite quantification. CRLB > 50% is not reliable and is a recommended cut-off by LCModel manual. Many in the field have used a CRLB lower than 35% as a standard. [53-55] Additionally, the CRLB should be kept in mind when interpreting the results.
6. Obtain GABA and Glx quantifications from the "DIFF" spectra, tCr from the "EDIT OFF" spectra, and tNAA from both "EDIT OFF" and "DIFF". Express concentrations of the different metabolites of interest as ratios over tCr. For GABA and Glx, multiply the ratio by the following group-averaged correction factor to account for the different basis set used for the numerator and denominator (tNAA from "EDIT OFF" spectra / tNAA from "DIFF" spectra).
   Note: Note: GABA and Glx concentrations can also be quantified using water or NAA signal.

Representative Results

Figure 6 shows the position of the VOI located on the representation of hand in M1 where all MRS measures were taken. In **figure 6D**, a 3D visualization shows a clear representation of the tDCS electrodes positioned on the scalp over the putative primary motor cortex. **Figure 7** shows representative “EDIT OFF” and difference (“DIFF”) spectra acquired in M1. Peaks corresponding to Glx, GABA+MM as well as NAA can be clearly seen.

Figure 8 shows the percentage of change between the MRS acquisition pre-tDCS and post-tDCS for the three different conditions in a single participant. Results from the post-tDCS session are separated into two time points to illustrate the evolution of change over time. **Figure 8a** shows the percentage of change for Glx. For sham stimulation, Glx concentration displays no notable modulation. For bilateral stimulation 1 (left anodal, right cathodal), again no notable modulation of Glx is observed; however, modulation of the concentration over time is opposite to what is observed in the sham stimulation. Finally, regarding bilateral stimulation 2 (left cathodal, right anodal), a similar pattern is observed to the sham stimulation but with a notable enhancement of the Glx concentration in the second time-point following stimulation.

Figure 8b shows the percentage of change in the concentration of GABA in relation to the condition of stimulation. For the sham stimulation, GABA concentration displays no notable modulation. However, a slight reduction is observed at both time points. The modulation of GABA following the sham stimulation is more important than for the Glx,. In contrast, a notable increase of GABA concentration is seen in the second-time point after bilateral stimulation 1 (left anode, right cathode). Finally, a similar pattern of change to the sham stimulation is observed for bilateral stimulation 2 (left cathode, right anode).

Figure 9 shows the obtained spectra from two different participants. **Figure 9a** shows a spectrum of good quality with an acceptable lipids signal. **Figure 9b** shows a spectrum with large lipids signals, which was excluded after visual inspection. Finally, **figure 10** shows displacement of the location of the voxel of interest following 5 mm participant movement.

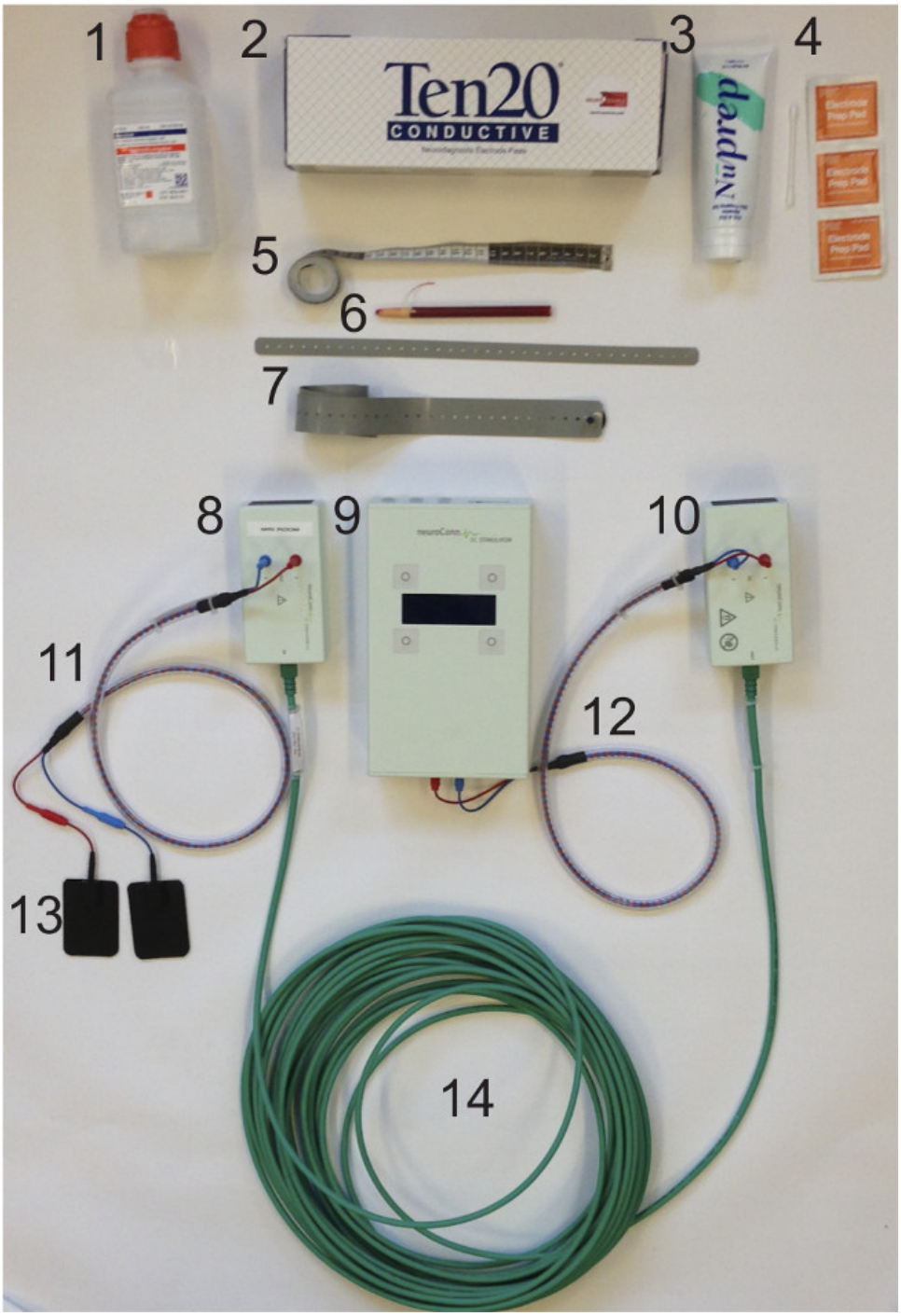

Figure 1: Materials. 1) Saline solution; 2) Conductive paste; 3) Electrode gel; 4) Alcohol prepping pad; 5) Measuring tape; 6) EEG pencil; 7) Rubber bands; 8) Inner box; 9) tDCS device; 10) Outer box; 11) Inner box cable; 12) Outer box cable; 13) Electrodes; 14) Long box cable

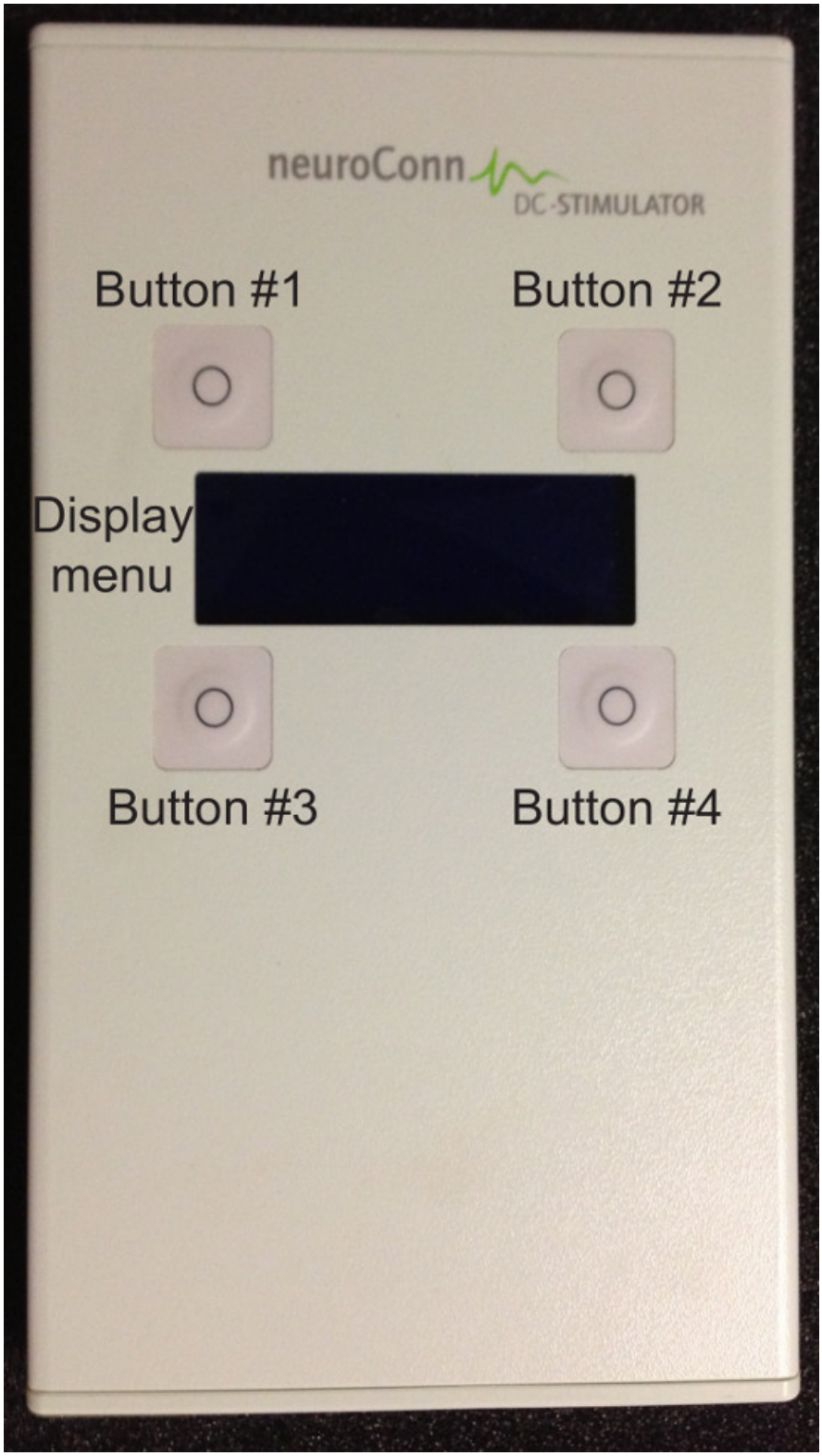

Figure 2: tDCS device Image of the positioning of the buttons on the specific tDCS device used in the present protocol. These buttons are used to pre-set the different settings.

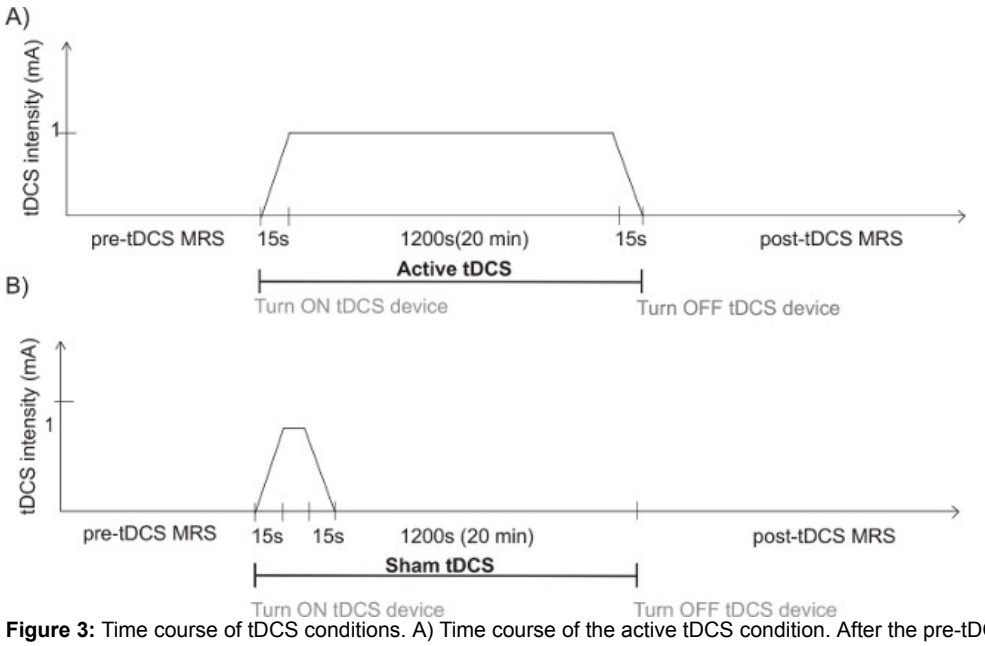

Figure 3: Time course of tDCS conditions. A) Time course of the active tDCS condition. After the pre-tDCS metabolite acquisition, turn on the tDCS device and ramp-up the current for 15 sec until an intensity of 1 mA is reached. Stimulate for 20 min and ramp-down the current for 15 sec until an intensity of 0 mA is reached. Do not turn off the tDCS device and proceed to the post-stimulation metabolite acquisition. B) Time course of the sham tDCS condition. After the pre-tDCS metabolite acquisition, turn on the tDCS device and ramp-up the current for 15 sec until an intensity of 1 mA is obtained. Stimulate for 15 sec (the minimum time available on the current device) and ramp-down the current for 15 sec until an intensity of 0 mA is reached. Wait for 20 min. Do not turn off the tDCS device and proceed to the post-stimulation metabolite acquisition.

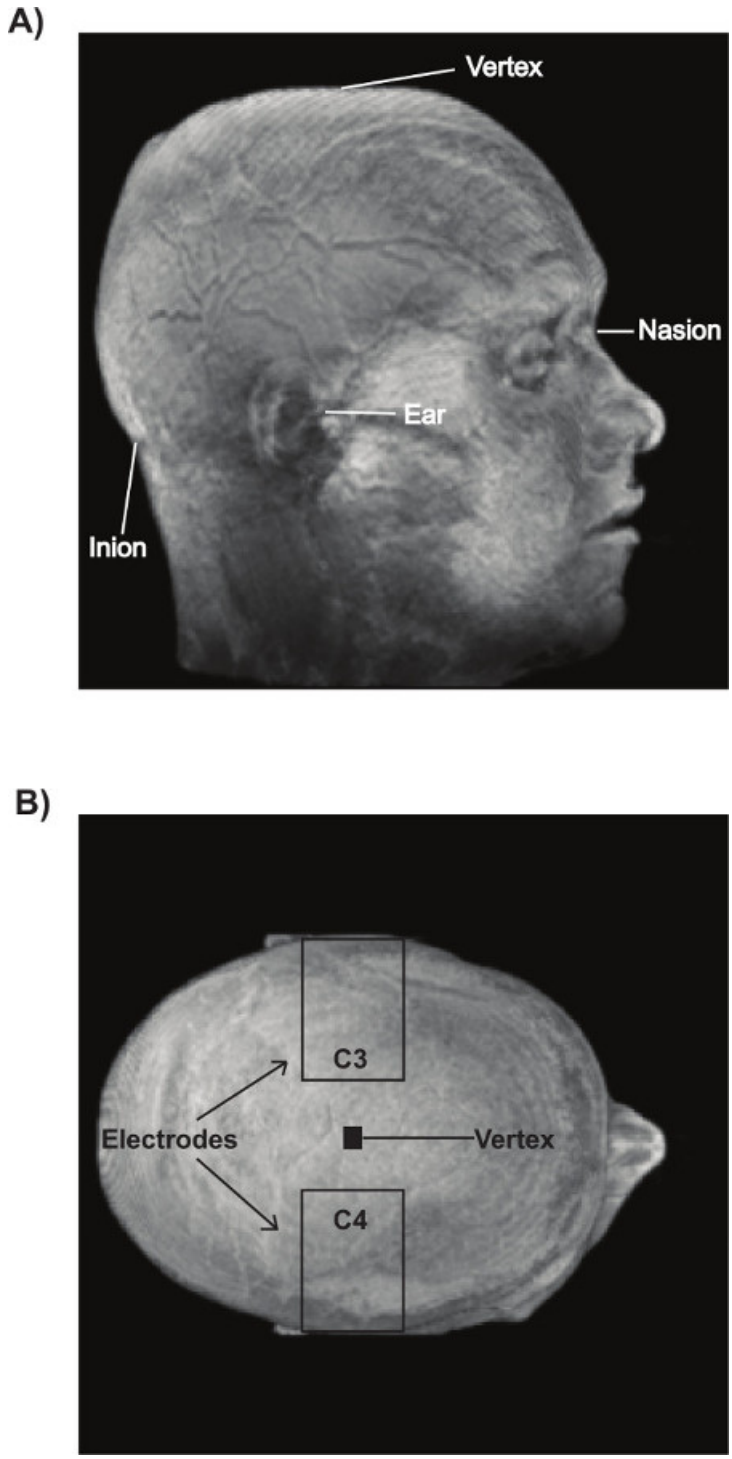

Figure 4: Electrode positioning A) 10/20 international system landmarks used for the identification of C3 and C4. The vertex (Cz) corresponds to 50% of the distance between the nasion and the inion, and 50% of the distance between the two preauricular points. B) C3 and C4 correspond to 20% of the total distance between the preauricular points, measured from the vertex point. Make sure to leave at least 8 cm of distance between both electrodes.

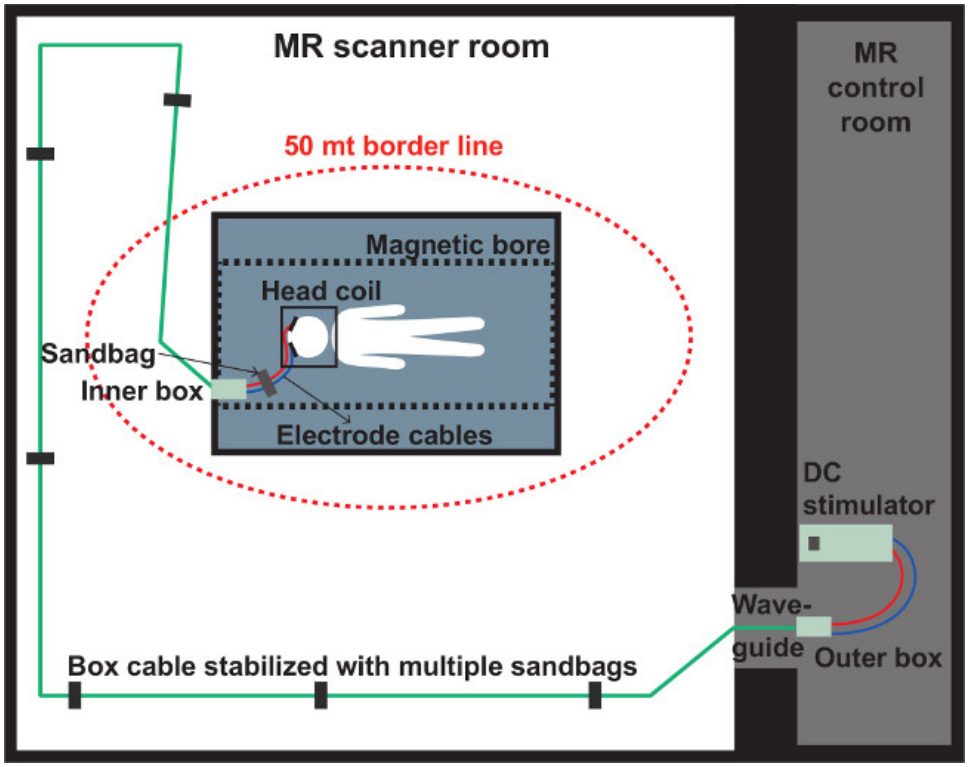

Figure 5: Schematic view of the MR room. Placement of the materials in the MR scanning and console rooms. It is essential to follow the protocol for the positioning of the different parts of the device in order to obtain a MR signal of good quality and for safety purposes.

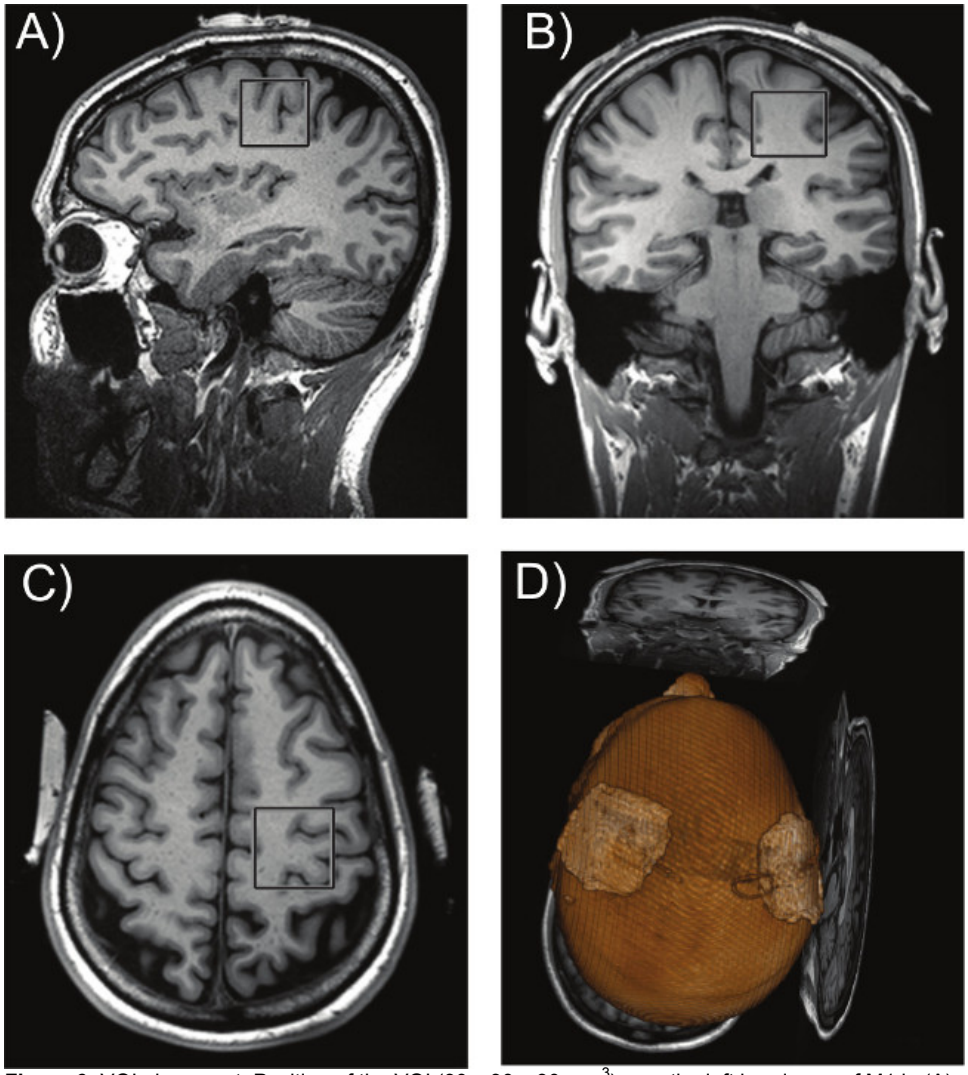

Figure 6: VOI placement. Position of the VOI (30 x 30 x 30 mm^3) over the left hand area of M1 in (A) sagittal, (B) coronal, and (C) axial slices. 3D visualization of the positioning of the electrodes is shown in (D).

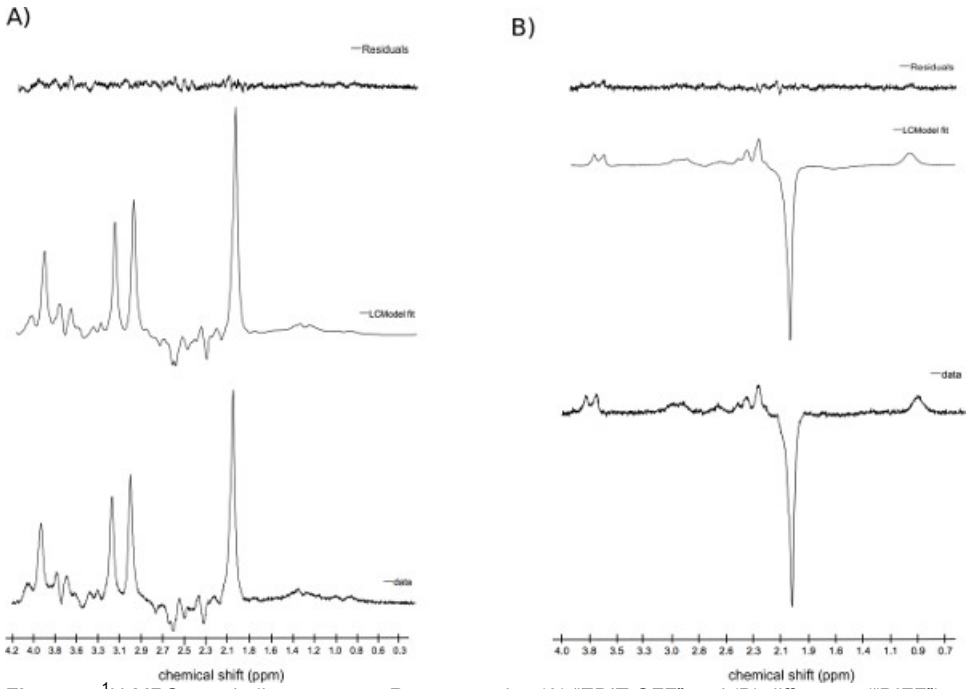

Figure 7: ^{1}H-MRS metabolite spectrum. Representative (A) "EDIT OFF" and (B) difference ("DIFF") spectra acquired with the MEGA-PRESS sequence [44,45] including the raw data, the fit from LCModel and the residuals. Cr: total creatine (creatine + phosphocreatine (Cr-CH_3 + PCr-CH_3)); NAA: *N*-acetyl-aspartate + NAAG (sNAA + NAAG); Glx : glutamate + glutamine (Glu + Gln); GABA + MM: γ-aminobutyric acid + macromolecules

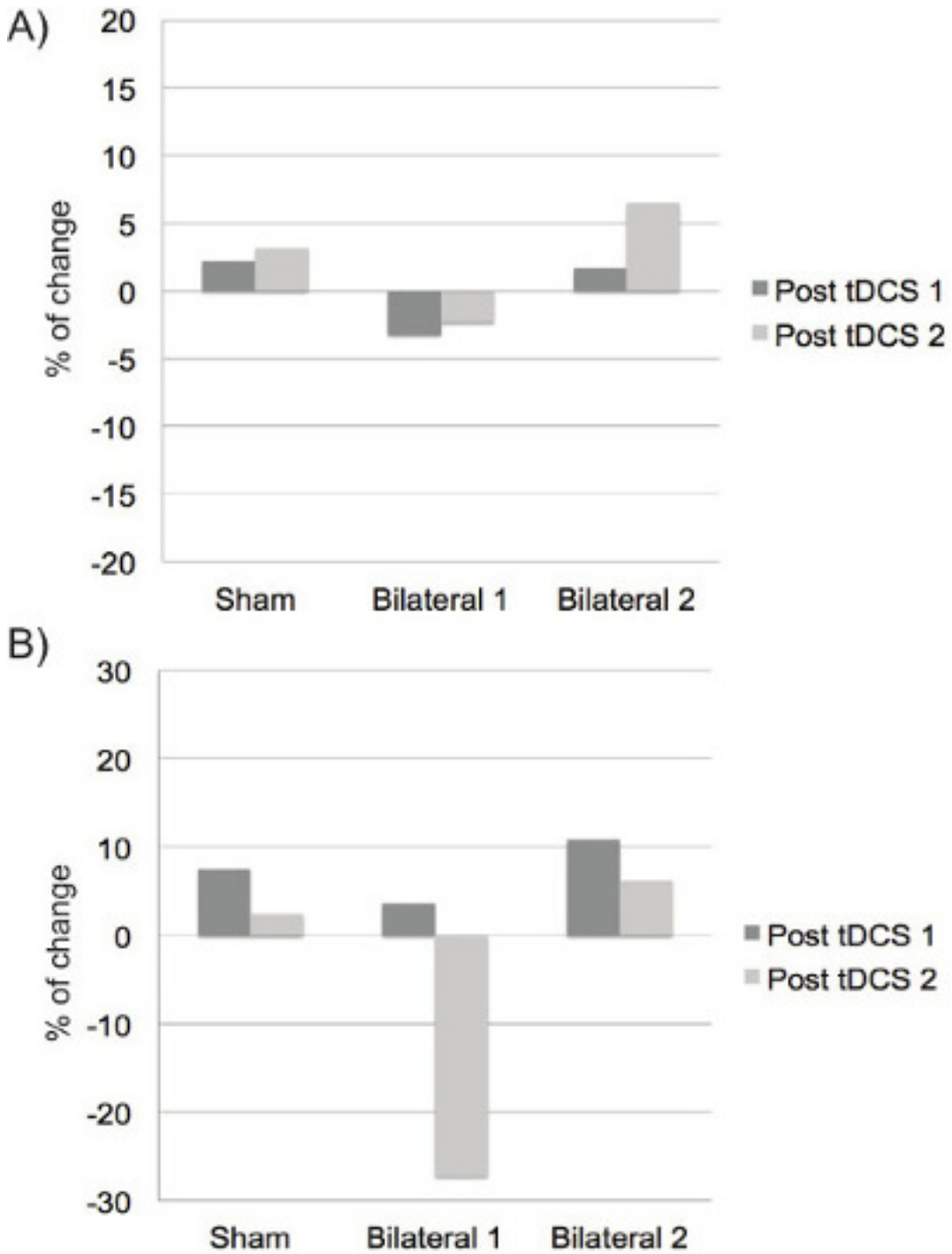

Figure 8: Effects of bilateral tDCS on Glx and GABA for a single subject. A) tDCS effects on Glx concentration are shown for the three conditions. Results are expressed as percentage of change between the pre-tDCS acquisition and the two post stimulation acquisitions. B) tDCS effects on GABA concentration are shown for the three conditions. Results are expressed as percentage of change between the pre-tDCS acquisition and the two post stimulation acquisitions. Sham: Bilateral, Bilateral 1: left anode, right cathode; Bilateral 2: left cathode, right anode

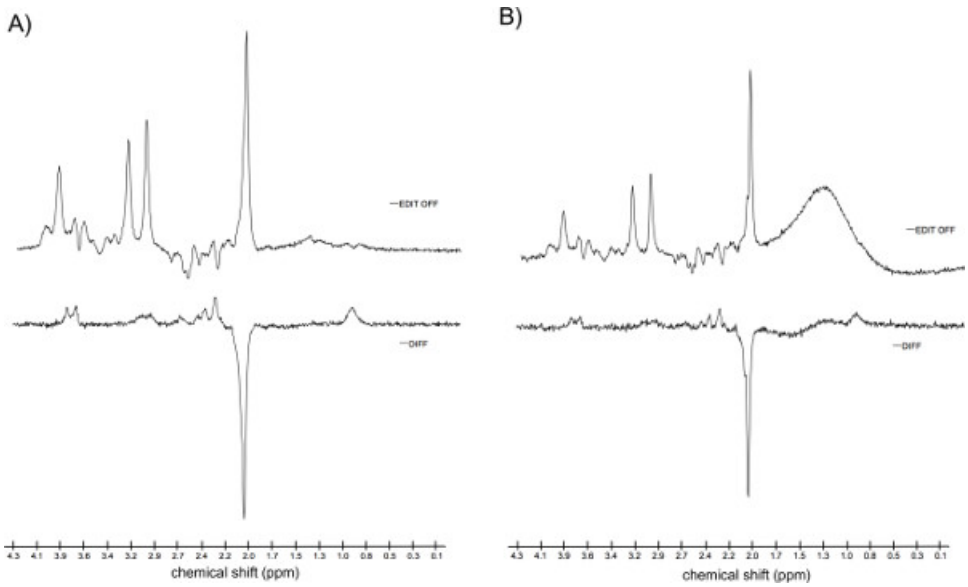

Figure 9: Visual inspection of the spectra A) Example of a good quality data. The figure shows the "EDIT OFF" and "DIFF" spectra with an acceptable amount of lipids. SNR from analysis of "DIFF" spectra: 56 CRLB of the GABA signal: 14% Lw of tCr-CH_3 at 3 ppm: 5.6 Hz. B) Example of a poor quality data caused by excessive movement of the participant. The figure shows the "EDIT OFF" and "DIFF" spectra. SNR from analysis of "DIFF" spectra: 39 CRLB of the GABA signal: 47% Lw of tCr-CH_3 at 3 ppm: 4.4 Hz

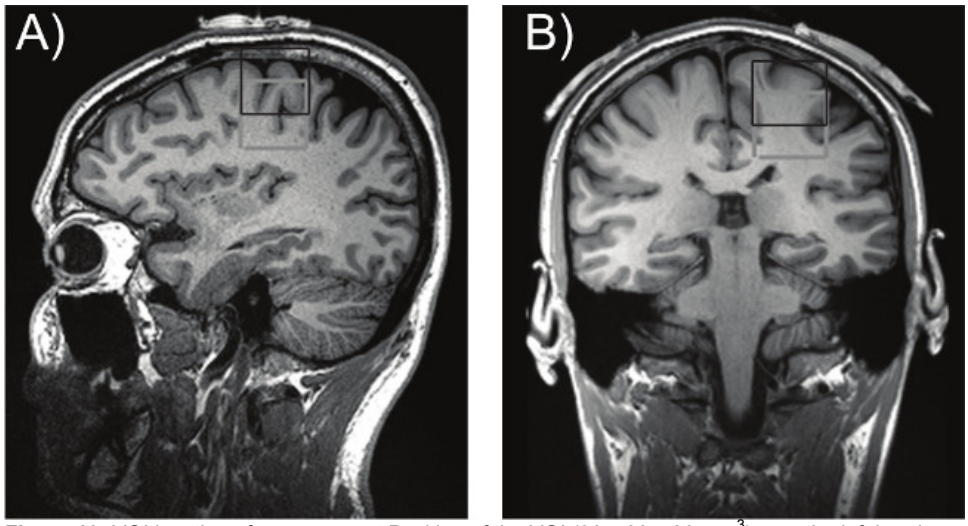

Figure 10: VOI location after movement Position of the VOI (30 x 30 x 30 mm^3) over the left hand area of M1 in (A) sagittal and (B) coronal slices after a movement of 5 mm. Inclusion of the cranial bones and the meninges in the box would lead to inclusion of lipids and elimination of the scan. The light grey square shows the initial position of the VOI.

Discussion

The present paper aimed to describe a standard protocol for combining tDCS and ^{1}H-MRS using a 3 T scanner. In the next section, methodological factors will be discussed.

Critical Steps

Contraindications Screening

Previous to the experiment, it is crucial to screen participants for any contraindication regarding the use of tDCS and ^{1}H-MRS. The use of the following exclusion criteria is recommended for tDCS: a psychiatric or neurological history, the presence of a pacemaker, a piece of metal implanted in the skull, a history of fainting, a history of seizures or a history of substance abuse. Because only metabolites from the left M1 will be acquired, the exclusion of left handed participants from the study is recommended. In fact, a recent study has shown differential interhemispheric inhibition between the dominant and non-dominant hemispheres depending on the hand preference, which could modulate the effect of stimulation [15]. Moreover, before starting the experiment, check for any lesion on the scalp and ask for any skin disease [56]. If there is a lesion present, try to avoid stimulating directly the affected area. It is also recommended to inspect the skin after stimulation [57]. Also, screen for the presence of allergies to any of the products used for electrode montage. For ^{1}H-MRS, the exclusion criteria should be the same as for any magnetic resonance imaging study including a careful screening of any prior surgeries for the presence of metal in the body.

It is also important to determine if the participant felt any discomfort during tDCS stimulation. Again, after the experiment, participant should be asked about any side effects. It is possible to use a record-form including the most reported side effects to quantify their presence in relation to the protocol (see [58] for an example). The most reported side effects are mild tingling (70.6%), moderate fatigue (35.3%), a light sensation of itching under the electrodes (30.4%), and slight burning sensation (21.6%) [58].

Movement Artefacts Reduction

Movement of the participant in the scanner is a major issue during ^{1}H-MRS as this is one of the main factors affecting the quality of the data [59]. As shown in **figure 10**, a movement of the subject (from 1 mm to 5 mm) can lead to large lipids signal in the spectrum thus altering the quality of the data and consequently, to the exclusion of this acquisition from the data. Therefore, it is crucial to carefully explain to the participant the importance of head stability during the entire scan. During the positioning of the participant in the scanner, it is important to ask the subject to find the most comfortable position to avoid any further movement. During positioning of the VOI, it is also important to notify the participant that even though the scan is silent, it is essential to remain still.

In addition, the duration of the experiment is an important factor to help minimize total amount of movement. First, it is important to use an optimal length for the anatomical sequence, as short as possible, but long enough to obtain good quality images for placement of the VOI. Second, the use of a short sequence of metabolite acquisition is recommended before tDCS. Third, in order to capture the temporal course of stimulation effects, the use of a longer sequence of acquisition after stimulation is advised. Fourth, compare pre- and post-experiment localizer images to estimate participant movement.

Analysis

The MEGA-PRESS sequence[44,45] is used to acquire localized, water suppressed, and edited spectra. A spatial localization in PRESS is performed using a 90° Hamming-filtered sync pulse (bandwidth time product = 8.75, duration = 2.12 msec, bandwidth (FWHM) = 4.2 kHz) and two 180° mao pulses (duration = 5.25 msec, bandwidth = 1.2 kHz). All localization pulses are executed at 3 ppm. A selective double-banded 180° Shinnar-Le Roux pulse is applied at 1.9, the resonance frequency of β–CH_2 of GABA, and 4.7 ppm alternating with 7.5 and 4.7 ppm. Additional water suppression using variable power with optimized relaxation delays (VAPOR) and outer volume suppression, OVS[50] were adapted for the human 3 T system and incorporated prior to MEGA-PRESS and are used to suppress water and to improve the localization of the VOI. When the selective pulse is applied at 1.9 ppm, the resonance at 1.9 ppm and the resonances within the bandwidth of the pulse are inverted causing refocusing of γ–CH_2 resonance of GABA ("EDIT ON"). When the selective pulse is applied at 7.5 ppm, the usual spectrum at T_E of 68 msec is obtained ("EDIT OFF") with the γ–CH_2 resonance of GABA phase modulated. The subtraction of signals from alternate scans results in selective observation of outer lines of GABA triplet and cancelation of the total creatine (creatine + phosphocreatine) resonance ("DIFF"). Due to the bandwidth of the inversion pulse, additional resonances of NAA, Glu + Gln, and macromolecules are also observed. The whole protocol is divided into four interleaved acquisitions and the frequency is updated before each individual scan to minimize the frequency drifts due to the hardware. The interleaved acquisition and single FID storage allows the correction of frequency and phase in post-processing.

The analysis method described in the protocol allows the calculation of the best fit of the experimental spectrum as a linear combination of model spectra. Model spectra in the basis set for "EDIT OFF" spectra were simulated based on density matrix formalism [59] and known chemical shifts and *J* couplings [60], and included the following: acetyl moiety of *N*-acetylaspartate (sNAA), alanine (Ala), ascorbate (Asc), aspartate (Asp), aspartate moiety of NAA (mNAA), CH_2 group of Cr (Cr-CH_2), CH_3 group of Cr (Cr-CH_2), CH_2 group of PCr (PCr-CH_2), CH_3 group of PCr (PCr-CH_2), GABA, glucose (Glc), Glu, Gln, glycerophosphorylcholine (GPC), glycine (Gly), glutathione (GSH), lactate (Lac), *myo*-inositol (mI), *N*-acetylaspartylglutamate (NAAG), phosphorylcholine (PCho), phosphorylethanolamine (PE), *scyllo*-inositol (sI), and taurine.

The basis set for "DIFF" spectra was generated from experimentally measured spectra of four 100 mM solutions of NAA, GABA, Glu, and Gln (600 ml spherical glass flasks) using the same parameters and scanner as for *in vivo* experiments. Each solution additionally contained K_2HPO_4 (72 mM), KH_2PO_4 (28 mM), sodium azide (0.1 mM), 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (TSP; 2 mM), formate (200 mM; optional), and distilled water. The basis set spectra were acquired at the physiological temperature of 37°C and every effort was made to minimize cooling (~1°C within the 15 of acquisition) by preheating the phantoms in a large water tank before placing each one in a smaller water-filled isolated plastic container, which was placed in the coil. Temperature and pH are particularly important in spectroscopy because they affect the chemical shift of the metabolites. Additionally, for both "EDIT OFF" and "DIFF" spectra, basis sets included a metabolite-nulled macromolecular spectrum experimentally measured from 10 subjects from the occipital cortex using the inversion-recovery (inversion time, T_I = 760 msec) technique using the same parameters as the regular MEGA-PRESS acquisition (except for T_R = 2.7 s) [61].

Phantom Testing

Testing the procedure on a 100 mM GABA phantom with and without the tDCS stimulator that will be used on participants with the exact scanner and sequence parameters is strongly advised prior to the first participant being studied. The procedure should include a localizer sequence, an anatomical sequence (i.e. MPRAGE), a line-width scan and 16 "EDIT ON" and "EDIT OFF" scans. This should be repeated if stimulator, stimulation parameters or scanners are changed. In order to investigate the presence of artefacts on the signal, one should review spectra for changes in SNR with and without the tDCS simulator, presence of spikes and noise at certain frequencies, and the SNR values and any important artefact on the anatomical images.

Possible Modifications to the Protocol

^{1}H-MRS Parameters

To acquire metabolite concentrations using ^{1}H-MRS, it is necessary to localize a specific region and excite signals in this volume [35]. In the present paper, the procedure for the placement of a single VOI over left M1 was described. However, many different modifications to this protocol can be applied. Successful measurement of metabolite concentrations have been demonstrated in various cortical and subcortical regions, such as the prefrontal cortex [62], hippocampus [63], cerebellum striatum and pons [64], visual cortex [66], and auditory cortex [67]. The size of the VOI can also differ as a function of the region of interest, but the volume typically ranges between 3 and 27 cm^3 [68]. However, it is hard to obtain concentration of low-concentration metabolites such as GABA from voxels smaller than 20 cm^3. An important issue is to make sure to avoid any contact of the VOI with the cranial bones, meninges, and extra-cerebral cerebrospinal fluid. In smaller brains, the VOI might include part of the left lateral ventricle. In this case, the inclusion of the ventricle is preferable over the inclusion of cranial bones.

Additionally, depending on the selected acquisition sequence, different metabolites can be quantified [69]. Previous methods, such as the Point-RE-Solved spectroscopy (PRESS) sequence [70] and stimulated echo acquisition mode (STEAM) [71], did not allow quantification of GABA at 1.5 T. However, because of the polarity-specific effect of tDCS on cortical excitability, the quantification of both excitatory (glutamate) and inhibitory (GABA) neurotransmitters is essential. In the present protocol, the use of the MEGA-PRESS spectral editing sequence [44,45] was shown, which allows the quantification of the major neurochemicals, including GABA (see **figure 6**). Other sequences allowing GABA quantification, such as ultra-short TE MRS and *J*-resolved MRS, have been developed over the last few years (see [41] for a review).

Finally, since metabolite concentrations are usually expressed as a ratio in relation to another metabolite (relative concentration), the choice of the reference metabolite is highly important, and particularly so in studies employing clinical populations [69]. The most commonly used reference metabolites are tCr and NAA, as their concentrations are found to be relatively stable in the human brain. It should be noted it is also possible to use an absolute quantification of metabolites which requires referencing to either an external (e.g., phantom) or internal signal (e.g., water signal) [68]. The use of an internal water reference requires an additional step of tissue correction since the water concentration and relaxation properties differ between grey matter, white matter and cerebrospinal fluid (CSF).[72] The tissue correction can be performed either using the estimated tissue composition in the VOI of all participants or using subject specific tissue composition from segmentation [73]. Additionally, it should be noted that tDCS carries the theoretical risk of inducing oedema, which could have a minor impact on water concentrations. However, Nitsche and collaborators [74] directly assessed this specific concern and showed no evidence of oedema following tDCS on the frontal cortex. Consequently, the use of a water reference is considered a viable option.

tDCS Parameters
Different electrodes sizes can be used [9] depending on the region of stimulation and the desired focality of stimulation [75,76]. Da Silva and collaborators [56] provide a comprehensive description of the different types of electrodes that are currently available for tDCS. Furthermore, as described in the present paper, ^{1}H-MRS is a useful technique that can be used to verify the underlying mechanisms of action of specific tDCS protocols that have been shown to improve symptoms in different clinical populations. Electrode positioning and duration of stimulation can be modified to investigate the effects of these specific tDCS protocols, such as those one used in the treatment of pain, depression, tinnitus, Parkinson's, migraine, and alcohol abuse (see [77] for a description of the protocols). It should also be noted that if the impedance level is above 20 kΩ, the device will not stimulate and display an impedance error message on the screen. Different factors that can cause a high impedance include: 1) insufficient amount of conductive paste on the electrodes; 2) insufficient pressure on the electrodes; 3) bad contact with the scalp (caused by hair); 4) thickening of the scalp due to baldness; 5) problems with connections; 6) problems with wiring; (7) problems with stimulator; and 8) problems with electrodes.

It should also be noted that localization of primary motor cortex for tDCS could be made more precise. In the present protocol, the 10/20 EEG system is used, which may introduce slight misalignment between maximum electrical field projection and actual representation of M1 within precentral gyrus. One possible way to circumvent this issue is to use transcranial magnetic stimulation to precisely localize the hand representation in M1 through the TMS-induced muscular response. Availability of a TMS unit in the vicinity of the MR scanner may limit this possibility.

***Safety of tDCS and* ^{1}H-MRS**
Safety of tDCS
Multiple studies have shown that tDCS is a safe neuromodulation technique producing only minor adverse effects in both non-clinical and clinical populations [10]. In fact, no case of epileptic seizure has ever been reported following tDCS [10]. However, the safety of tDCS has yet to be investigated in children and pregnant women [78].

MR Compatible Materials
Caution should be taken when stimulating inside a MR scanner. All materials brought into the MR room must be MR compatible (see **figure 1**). Because of the possible interaction between the electric current produced by the tDCS and the MR scanner, tDCS should always be turned on, and the electrodes should remain connected, during the MR sequences described in the present protocol. Coiling of the wires under the head coil can produce artefacts and distortions in the signal. Moreover, improper connection of the wires could potentially produce a current strong enough to burn the participant [79]. Finally, it is important to never disconnect the electrodes while the current is flowing as this might cause an unwanted high-voltage stimulation.

tDCS-MRS Technique
Using tDCS in conjunction with MRS offers the possibility to better understand the mechanism underlying modulation of brain activity with this relatively new neuromodulation technique. However, some limitations of the technique should be addressed. First, the electrodes used in tDCS are usually rather large and the effects of stimulation are believed to cover a wide spatial extent of brain tissue. Coupled with the fact that MRS acquisition is limited to a small voxel of interest, tDCS-MRS only allows for the assessment of spatially circumscribed effects despite presumed widespread modulation of brain excitability. One possible way to circumvent this problem is to use multiple voxels of interest distributed throughout the brain. However, this will significantly increase duration of the experimental session, which is already a major limitation of the present technique. Indeed, when considering participant preparation, pre-tDCS MRS, tDCS intervention and post-tDCS MRS, a full session may easily last up to two hours. Duration can also increase if one wishes to map the time course of tDCS effects on metabolite concentration.

An important issue related to the duration of the experiment is the possibility that electrode impedance will increase after the participant is in the scanner. Since tDCS can easily begin more that 45 minutes after electrode placement, there is a risk that the stimulating electrodes will gradually lose adherence to the participant's scalp if paste application is not optimal and electrodes are not held tightly enough. If impedance reaches more than 20 kΩ, stimulation will not be possible and the participant will need to be removed from the scanner to solve the problem. Since the described procedure involves multiple scanning of the same area pre- and post-tDCS, removing the participant from the scanner may create important displacement of the voxel of interest. It is therefore very important to test impedance immediately prior to scanning and to take great care when installing electrodes.

Theoretically, the current flow of the tDCS could produce artefacts in the MR signal. Antal and collaborators [80] investigated this specific concern by measuring the impact of different tDCS conditions (with and without electrodes, with and without stimulation, etc.) on the quality of functional

magnetic resonance images. However, to our knowledge, the presence of artefacts in the spectroscopy signal due to the presence of the tDCS device in the scanner has yet to be assessed.

Finally, care should be taken with regards to the resistors in the electrode cables. The MR field may damage resistors, thus preventing stimulation. As a precautionary measure, resistance should be tested outside the scanner environment prior to every MRS session. In addition, an impedance of more than 20 kΩ can lead to skin reactions and high impedance may reflect an incipient or actual problem with the stimulator. Therefore, the stimulator should be checked carefully before every participant and impedance levels checked outside the scanner room prior to every MRS session.

Combined tDCS and ^{1}H-MRS is a powerful tool that provides a quantitative measure of the effect of clinically used treatments on brain metabolism. As the physiological mechanism of tDCS effects remains poorly understood, there is a need for multimodal approaches that can shed light on these processes. With the recent surge in interest in tDCS as a clinical tool for pathologies such as stroke [27,30,31] and depression [81], it is clear that combination of tDCS with MRS may be an important tool to better understand the therapeutic effects of tDCS. Furthermore, tDCS-MRS may serve as an early tool to determine which patients have a better chance to respond clinically to tDCS. If such a marker is found, tDCS-MRS may be used as a screening test prior to enrolling patients in a tDCS intervention.

Disclosures

The authors have nothing to disclose.

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