Video Article

Non-Invasive Electrical Brain Stimulation Montages for Modulation of Human Motor Function

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Abstract

Non-invasive electrical brain stimulation (NEBS) is used to modulate brain function and behavior, both for research and clinical purposes. In particular, NEBS can be applied transcranially either as direct current stimulation (tDCS) or alternating current stimulation (tACS). These stimulation types exert time-, dose- and in the case of tDCS polarity-specific effects on motor function and skill learning in healthy subjects. Lately, tDCS has been used to augment the therapy of motor disabilities in patients with stroke or movement disorders. This article provides a step-by-step protocol for targeting the primary motor cortex with tDCS and transcranial random noise stimulation (tRNS), a specific form of tACS using an electrical current applied randomly within a pre-defined frequency range. The setup of two different stimulation montages is explained. In both montages the emitting electrode (the anode for tDCS) is placed on the primary motor cortex of interest. For unilateral motor cortex stimulation the receiving electrode is placed on the contralateral forehead while for bilateral motor cortex stimulation the receiving electrode is placed on the opposite primary motor cortex. The advantages and disadvantages of each montage for the modulation of cortical excitability and motor function including learning are discussed, as well as safety, tolerability and blinding aspects.

Video Link

The video component of this article can be found at https://www.jove.com/video/53367/

Introduction

Non-invasive electrical brain stimulation (NEBS), the administration of electrical currents to the brain through the intact skull, can modify brain function and behavior[1-3]. To optimize the therapeutic potential of NEBS strategies understanding the underlying mechanisms leading to neurophysiological and behavioral effects is still needed. Standardization of application across different laboratories and full transparency of stimulation procedures provides the basis for comparability of data which supports reliable interpretation of results and evaluation of the proposed mechanisms of action. Transcranial direct current stimulation (tDCS) or transcranial alternating current stimulation (tACS) differ by parameters of the applied electrical current: tDCS consists of an unidirectional constant current flow between two electrodes (anode and cathode)[2-6] while tACS uses an alternating current applied at a specific frequency[7]. Transcranial random noise stimulation (tRNS) is a special form of tACS that uses an alternating current applied at random frequencies (*e.g.*, 100-640 Hz) resulting in quickly varying stimulation intensities and removing polarity-related effects[4,6,7]. Polarity is only of relevance if the stimulation setting includes a stimulation offset, *e.g.*, noise spectrum randomly changing around a +1 mA baseline intensity (usually not used). For the purpose of this article, we will focus on work using tDCS and tRNS effects on the motor system, closely following a recent publication from our lab[6].

The underlying mechanisms of action of tRNS are even less understood than of tDCS but likely different from the latter. Theoretically, in the conceptual framework of stochastic resonance tRNS introduces stimulation-induced noise to a neuronal system which may provide a signal processing benefit by altering the signal-to-noise ratio[4,8,9]. TRNS may predominantly amplify weaker signals and could thus optimize task-specific brain activity (endogenous noise[9]). Anodal tDCS increases cortical excitability indicated by alteration of the spontaneous neuronal firing rate[10] or increased motor evoked potential (MEP) amplitudes[2] with the effects outlasting the stimulation duration for minutes to hours. Long-lasting increases in synaptic efficacy known as long-term potentiation are thought to contribute to learning and memory. Indeed, anodal tDCS enhances synaptic efficacy of motor cortical synapses repeatedly activated by a weak synaptic input[11]. In accordance, improved motor function/skill acquisition is often revealed only if stimulation is co-applied with motor training[11-13], also suggesting synaptic co-activation as a prerequisite of this activity-dependent process. Nevertheless, causality between increases in cortical excitability (increase in firing rate or MEP amplitude) on one hand and improved synaptic efficacy (LTP or behavioral function such as motor learning) on the other hand has not been demonstrated.

NEBS applied to the primary motor cortex (M1) has attracted increasing interest as safe and effective method to modulate human motor function[1]. Neurophysiological effects and behavioral outcome may depend on the stimulation strategy (*e.g.*, tDCS polarity or tRNS), electrode size and montage[4-6,14,15]. Aside from subject-inherent anatomical and physiological factors the electrode montage significantly influences electric field distribution and may result in different patterns of current spreading within the cortex[16-18]. In addition to the intensity of the applied current the size of the electrodes determines the current density delivered[3]. Common electrode montages in human motor system studies include (**Figure 1**): 1) anodal tDCS as **unilateral M1 stimulation** with the anode positioned on the M1 of interest and the cathode positioned on the

contralateral forehead; the basic idea of this approach is upregulation of excitability in the M1 of interest[6,13,19-22]; 2) anodal tDCS as **bilateral M1 stimulation** (also referred to as "bihemispheric" or "dual" stimulation) with the anode positioned on the M1 of interest and the cathode positioned on the contralateral M1[5,6,14,23,24]; the basic idea of this approach is maximizing stimulation benefits by upregulation of excitability in the M1 of interest while downregulating excitability in the opposite M1 (*i.e.*, modulation of interhemispheric inhibition between the two M1s); 3) For **tRNS**, only the above mentioned **unilateral M1 stimulation** montage has been investigated[4,6]; with this montage excitability enhancing effects of tRNS have been found for the frequency spectrum of 100-640 Hz[4]. The choice of brain stimulation strategy and electrode montage represents a critical step for an efficient and reliable use of NEBS in clinical or research settings. Here these three NEBS procedures are described in detail as used in human motor system studies and methodological and conceptual aspects are discussed. Materials for unilateral or bilateral tDCS and unilateral tRNS are the same (**Figure 2**).

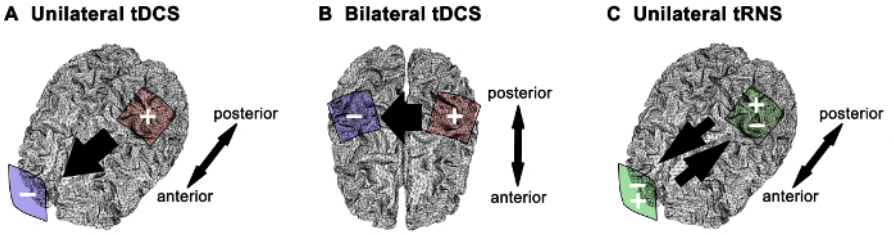

Figure 1. Electrode montages and current direction for distinct NEBS strategies. (**A**) For unilateral anodal transcranial direct current stimulation (tDCS), the anode is centered over the primary motor cortex of interest and the cathode positioned over the contralateral supra-orbital area. (**B**) For bilateral motor cortex stimulation, anode and cathode are located each over one motor cortex. The position of the anode determines the motor cortex of interest for anodal tDCS. (**C**) For unilateral transcranial random noise stimulation (tRNS), one electrode is located over the motor cortex and the other electrode over the contralateral supra-orbital area. The current flow between electrodes is indicated by the black arrow. Anode (+, red), cathode (-, blue), Alternating current (+/-, green). Please click here to view a larger version of this figure.

Protocol

Ethics statement: Human studies require written informed consent of participants before study entry. Obtain approval by the relevant ethics committee before recruitment of participants. Make sure studies are in accordance with the Declaration of Helsinki. The representative findings reported here (**Figure 4**) are based on a study performed in accordance with the Declaration of Helsinki amended by the 59th WMA General Assembly, Seoul, October 2008 and approved by the local Ethics Committee of the University of Freiburg. All subjects gave written informed consent before study entry[6].

1. Safety Screening

1. Screen the participant for potential contraindications for noninvasive brain stimulation[3], *e.g.*, by using questionnaires[25].

2. Motor Cortex Localization

1. Locate the participant's hand motor cortex by one of two distinct approaches, by locating the brain representation of the muscle of interest by transcranial magnetic stimulation (TMS)-induced MEP, or by locating the standard M1 position (C3/C4) based on the EEG 10/20 international system with a measuring tape[26].
2. For TMS-induced MEP recording ask the participant to remove any object that may be influenced by TMS magnetic field, including credit cards, mobile phones and metal objects in general.
3. Ask the participant to sit comfortably.
4. Verify connections between EMG amplifier and the computer used for signal configuration and acquisition when using a software interface.
5. Turn on the EMG amplifier and connect EMG electrode cables.
6. Clean participant's skin by softly rubbing with skin preparation paste in the regions of the hand where the electrodes will be placed. Remove excess with clean gauze pad.
7. Attach EMG surface electrodes in a belly-tendon montage on the hand muscle of interest (*e.g.*, M. abductor pollicis brevis of the right hand) and connect a ground electrode (*e.g.*, on forearm). The purpose of the study determines which hand muscle to use.
   Note: For reusable electrodes it is necessary to apply a small amount of conductive paste on the electrode surface before attaching it to the participant's skin.
8. (optional step) Start the recording software for MEP acquisition if MEP data storage is desired.
9. Check the EMG impedance values. Ensure that the impedance is < 20 kOhm.
10. Turn on the magnetic stimulator and charge the capacitor by pressing the corresponding "charge" button.
11. Place a figure-of-eight TMS coil on the participant scalp on the interhemispheric fissure and move it to the motor cortex area (around positions C3/C4 of the EEG 10/20 international system). Hold the TMS coil at a 45°-50° angle referenced to the interhemispheric fissure[27,28], with the handle oriented backwards, producing a cortical current flow from posterior to anterior[29].
    Note: Two distinct TMS coils are used for motor cortex localization: figure-of-eight or circular coils. If possible, use a figure-of-eight coil as it provides more focal brain stimulation[30] and greater reliability of measurements of cortical excitability[31].
12. When the magnetic stimulator is charged (visible on the display), discharge the stimulator either by pressing the trigger button or by stepping on the foot switch or automatically by a software program. This will subsequently deliver a single TMS pulse through the connected TMS coil placed over the participant's scalp. Default TMS pulse settings (*e.g.*, 100 µs rise time of the induced current and 800 µs decay time for monophasic stimuli; shorter decay times for biphasic stimuli) are specific to the device (firmware).

13. Start with low stimulation intensity (*e.g.*, set the intensity to 45% output using the stimulation intensity controller knob on the stimulator) and watch for MEPs visible on the EMG amplifier.
    1. If no MEP is visible increase the stimulation intensity in 2-5% steps until an MEP is clearly present (*e.g.*, 0.5-1 mV amplitude). Repeat stimulation by pressing the trigger button or activating the foot switch if pulse delivery is not automated. Inform the participant that stimulation will be slightly stronger and that limb movements, facial twitch and eye-blinks are expected.
    Note: Establish a minimum interval of 5 sec between pulses to avoid low-frequency stimulation effects on brain excitability.
14. Move the coil radially in 1 cm steps around the initially stimulated site to find the spot with the largest MEP response following the application of single TMS pulses. From there, start again moving the coil to secure the "hotspot" (cortical area with maximal MEP amplitude).
    Note: The use of a head cap (*e.g.*, used for grid markings) for the localization procedure is not recommended since the cap needs to be removed for NEBS electrode placement and the hotspot position may be lost.
15. Reduce the stimulation intensity in approximately 2%-steps using the stimulation intensity controller knob on the stimulator (MEP must still be present). This will avoid inaccuracy due to supramaximal stimulation. Reconfirm the hotspot by moving the coil radially in 1 cm steps around the hotspot and checking for MEP size. The hotspot should still correspond to the largest and most consistent MEP amplitude.
    Note: Ask the participant to voluntarily contract the muscle of interest if the hotspot is difficult to find (*e.g.*, no MEP present at high stimulation intensities). By doing so, the stimulation intensity needed to elicit MEP is decreased and it may be easier to identify relevant cortical stimulation sites. If this method is used, ask the participant to relax the muscle after finding a relevant stimulation site and adjust stimulation intensity so that reliable MEPs can be found when the muscle is at rest. Proceed to find the hotspot.
16. Mark the hotspot position and coil orientation with non-permanent skin marker.
17. For bilateral M1 stimulation, repeat steps 2.11 to 2.16 for the contralateral limb.

3. NEBS Electrode Preparation

1. Connect cables to rubber electrodes, and place the electrodes inside the sponge bags. Make sure electrode size and sponge bag size do match. Materials are commercially available in standard sizes (*e.g.*, 5x5 cm^2, 5x7 cm^2).
2. Soak sponge bags on both sides with isotonic NaCl solution, but avoid excessive soaking to prevent salt bridges or dripping onto the volunteer.
    1. This step is optional: To prevent leakage of NaCl solution when using bandages instead of rubber bands, place the electrodes and sponge bags inside non-conductive rubber sponge covers.
    Note: Alternatively, cover the rubber electrode with conductive paste and place them directly on the participant's head, *i.e.,* not using sponge bags or rubber sponge covers.

4. NEBS Electrode Placement (Figure 1)

1. Find the head marking(s) indicating the motor cortical hotspot and separate the hair around the area.
2. To improve conductance clean the skin before electrode placement by gently rubbing the skin area around the head markings with a swab soaked with 40-50% alcohol or skin preparation paste. Do not scratch the skin! Remove excess with a swab and clean area again with isotonic NaCl solution. Dry the area afterwards.
    Note: Make sure the head marking(s) remain visible; remark if needed.
3. Place one electrode following the head marking for the M1 of interest (contralateral to the hand of interest). Bring the sponge as much as possible in direct contact with the skin. Place the electrode cable towards the participant's back to avoid disturbance during stimulation and/or task execution and to ease connection to the NEBS device.
    Note: The hair below the electrode should get damp. In case of excessive hair moistening, use paper or hand towels to absorb the excess.
    Note: For anodal tDCS, the electrode placed on the motor cortical hotspot of interest (increase of excitability is desired) corresponds to the anode, usually connected to the red cable. The cathode (usually connected to a black or blue cable) is placed on the opposite supraorbital area or M1 (see below). Conventionally, electrode placement is the same for tRNS, although in the classical protocol there is no polarity specificity due to the alternating current flow. Specific placement may be important if the stimulation settings include a stimulation offset.
4. For **unilateral M1 stimulation** place the second electrode (for anodal tDCS: the cathode) over the contralateral supra-orbital area (corresponding to electrode Fp2 in the EEG 10/20 international system). Make sure the cable is oriented towards the back of the participant.
5. For **bilateral M1 stimulation** skip step 4.4. Place the second electrode (for anodal tDCS: the cathode) on the opposite M1 following the head marking ipsilateral to the limb used in the study. Make sure the cable is oriented towards the back of the participant.
6. Cover the head twice with an elastic bandage circularly in the medio-lateral direction to stabilize the M1 electrode, then use the remaining bandage to cover the head circularly in the anterior-posterior direction to stabilize both electrodes.
7. Use an adhesive tape to fix the end of the bandage.
8. Secure the cables with an adhesive tape on the participant's neck or shirt.
9. Connect electrode cables to the NEBS device.

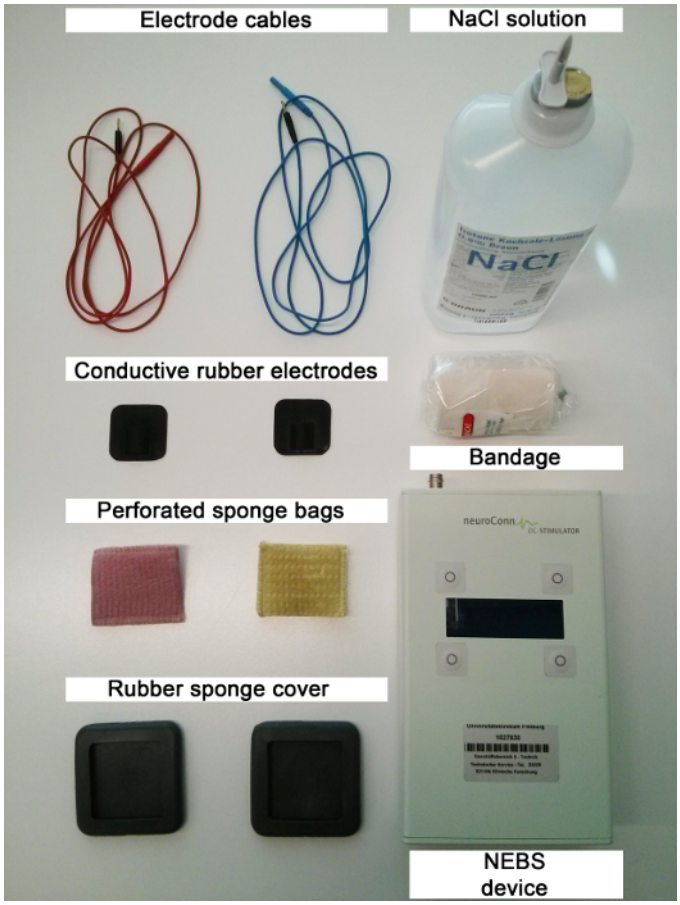

Figure 2. Materials used for NEBS protocols. Conventional materials used in non-invasive electrical brain stimulation protocols include an NEBS device, electrode cables, conductive rubber electrodes, perforated sponge bags, rubber sponge cover (optional), isotonic NaCl solution and bandages. Please click here to view a larger version of this figure.

5. Stimulation

1. Switch on the NEBS device.
2. Adjust NEBS device settings regarding stimulation type (tDCS or tRNS), intensity (*e.g.*, 1 mA, 1.5 mA or 2 mA), duration (*e.g.*, 10-40 min), ramping up and down (time between beginning of stimulation and maximum intensity, typically 8-15 sec), and additional factors related to stimulation type (*e.g.*, frequency spectrum for tRNS).
   Note: Conventionally, sham stimulation includes ramping up immediately followed by ramping down. Accordingly, the participant has the sensation of the stimulation but the duration of the stimulation is not sufficient to exert lasting effects on brain function. Some NEBS devices include a study mode which allows blinding of participant and investigator by entering a study specific subject code. The code determines stimulation settings automatically. Alternatively, a second experimenter may set the stimulation settings in each session and cover the display from the experimenter conducting the stimulation.
3. Inform the participant about potential side effects associated with NEBS. Common adverse effects include skin itching/tingling or burning sensation underneath the electrodes, headache, and discomfort[32]. Burning sensation may be a sign of poor electrode contact with the skin.
4. Start the stimulation.
   Note: Common stimulation duration lasts approximately 10-20 min based on reports investigating changes on cortical excitability (see representative results section). Empirically, the maximum stimulation duration was set to 40 min[3].
5. Check for continuity of stimulation during ramping up and stimulation. If impedance is too high or electrodes are in bad contact with the skin, the stimulation may terminate automatically.
   Note: In case the impedance is too high or the participant reports increasing discomfort during the stimulation try to decrease impedance by, *e.g.*, better fixating the electrodes at the stimulation sites or adding conductive medium. NaCl solution may be added by using a syringe directly in the sponges after their placement on the head.
   Note: For safety reasons some devices report the impedance throughout the stimulation. The NEBS device may shut off if impedance reaches a specific threshold (*e.g.*, 55 kOhms).
6. If NEBS is co-applied with execution of a motor task, start the testing/training after stimulation is ramped up and the participant is feeling comfortable with stimulation. In case the study does not include a motor task during stimulation, make sure the participant remains seated and awake during the stimulation period, and wait until stimulation is over.
7. Check with the participant for side effects of the stimulation, *e.g.*, by handing out a standardized questionnaire[32] or directly asking the participant. In case of studies including multiple days of stimulation, take note of any possible side effects between days.

Note: For assessment of blinding efficacy, ask the participant after each stimulation session to guess which stimulation type (sham/condition) the participant underwent. If the experimenter is also blinded, the experimenter could also note his guess regarding the participant's stimulation type. Compare answers with the actual stimulation type to verify rate of correct guesses[33].

8. Disinfect electrodes and sponges with non-hazardous substances such as 40-50% alcohol. Thoroughly rinse in water afterwards. Let materials dry before storing.

Representative Results

To investigate the effects of NEBS on the human motor system it is important to consider appropriate outcome measures. One advantage of the motor system is the accessibility of the cortical representations by electrophysiological tools. Motor evoked potentials are frequently used as an indicator of motor cortical excitability. After application of 9 or more minutes of anodal tDCS at a current density of 29 μA/cm^2, motor cortical excitability is increased for at least 30 min in the majority of healthy volunteers[19,21,22] (see also **Figure 3**). Cathodal tDCS mostly causes the opposite (excitability-decreasing) or no effect[19,22]. However, as discussed recently[22], there is some variability in the response direction, with some subjects showing the opposite direction of effect for anodal and cathodal tDCS. This should be taken into account for sample size calculations in studies using NEBS. Interestingly, comparable changes in M1 excitability were found after unilateral and bilateral tDCS[5,23], and simple motor function was similarly improved directly after each stimulation type[5]. Therefore it is currently under investigation whether additional down-regulation of excitability of the contralateral M1 using the bilateral M1 montage exerts specific benefits to motor behavior (see below). In contrast, resting state fMRI indicated clearly different cortical network changes: bilateral tDCS modulates functional connectivity in the primary and secondary motor and in prefrontal areas, while unilateral tDCS modulates functional connectivity in prefrontal, parietal and cerebellar areas[34].

tRNS has just recently developed as a tool to modulate cortical excitability[4]. Due to the alternating current tRNS is applied without polarity specificity (as long as there is no offset of stimulation intensity). However, efficiency of tRNS seems to depend on the applied noise spectrum, with high frequencies (100-640 Hz) showing more robust effects than low frequencies (<100 Hz)[4]. When directly compared to unilateral anodal tDCS, a similar but slightly longer lasting increase of M1 excitability (measured by MEP amplitude changes) was found after unilateral tRNS (**Figure 3**).

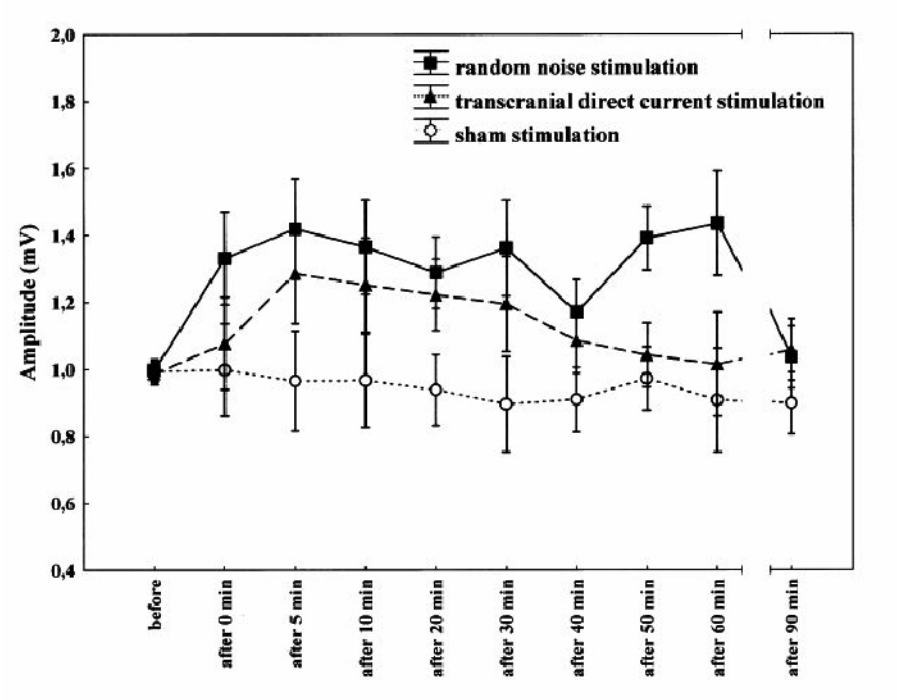

Figure 3. Time course of motor cortical excitability after different NEBS strategies. The MEP amplitude is shown as a function of time before and after 10 min of unilateral anodal transcranial direct stimulation (tDCS) or transcranial random noise stimulation (tRNS) applied to the primary motor cortex at a current density of 29 μA/cm^2 (1 mA / 35 cm^2). Error bars indicate standard error. Note that tRNS exerts similar effects on motor cortical excitability compared to anodal tDCS. MEP amplitude returns to baseline levels after approximately 50 min for anodal tDCS and after 90 min for tRNS. From Terney *et al.* (2008)[4] with permission. Please click here to view a larger version of this figure.

Despite the heterogeneity of study designs, a common concept starts to evolve from NEBS trials testing the effects of tDCS and tRNS on motor function: NEBS influences motor performance or skills when simultaneously applied with training/testing. Anodal tDCS and tRNS applied as unilateral M1 stimulation or anodal tDCS applied as bilateral M1 stimulation during training were all shown to improve implicit motor sequence learning[4,35-38] on the serial reaction time task[39]. Similarly, unilateral anodal tDCS applied during motor training was shown to increase rate of learning in an explicit motor learning paradigm[40]. However, effects of cathodal stimulation on implicit and explicit motor learning seem to be different: while cathodal tDCS during training did not significantly affect sequence learning during implicit motor learning[35], it was reported to negatively affect explicit motor learning[40]. The reasons for this discrepancy need further investigation.

In previous investigations focusing on more complex motor skill learning over multiple days anodal tDCS applied as unilateral M1 stimulation during training significantly enhanced visuomotor skill learning[13,20]. Skill was determined by changes in movement accuracy as a function of movement speed (*i.e.*, the speed-accuracy-tradeoff). Strikingly, in a direct comparison of electrode montages and stimulation types, both unilateral and bilateral M1 anodal tDCS and unilateral tRNS all enhanced skill learning on a visuomotor word and letter tracing task[6] (**Figure 4A**). With regard to the mechanisms, it is currently unknown whether tDCS and tRNS operate by the same mechanisms of action. However, the time course of skill gains within session clearly differed between tDCS and tRNS: Unilateral tDCS exerted major effects on skill gains immediately after stimulation started. In contrast, bilateral tDCS and tRNS slowly enhanced skill gains during sessions (**Figure 4B**). This divergence points to temporally specific interactions between the NEBS type and the motor learning process. This should be considered when choosing stimulation types for future investigations of the motor system in healthy subjects as well as patients with neurological disorders.

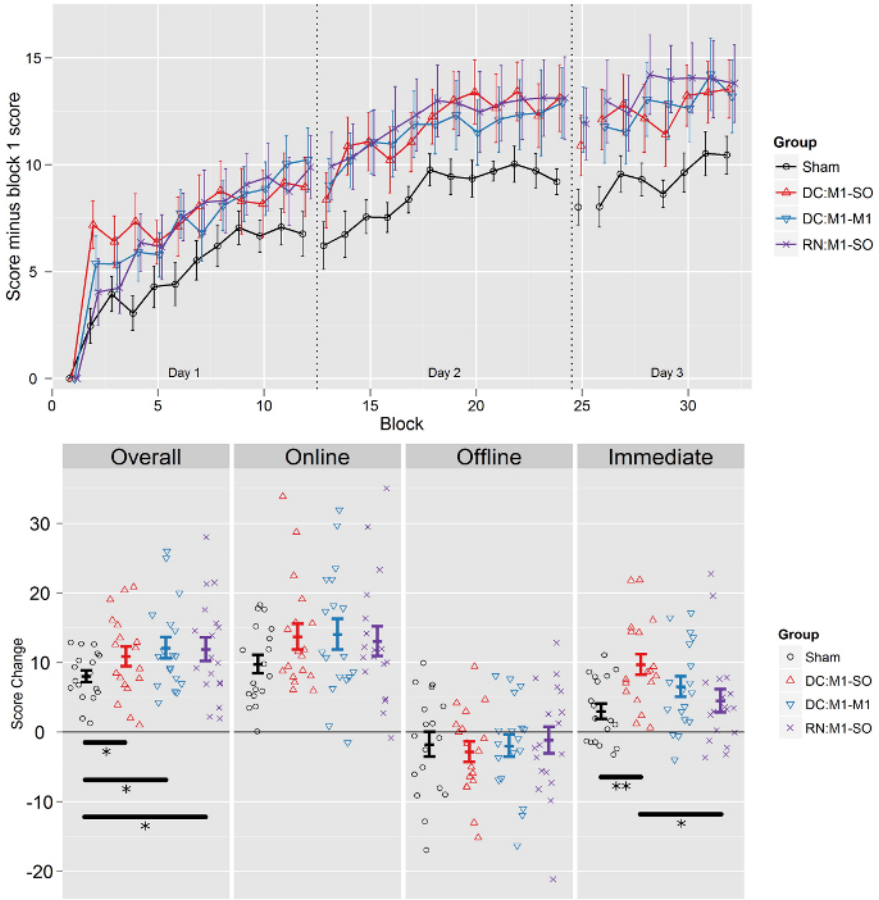

Figure 4. Enhancement of motor skill by training and augmentation by different NEBS strategies. (**A**) Changes in motor skill during three days of motor training per stimulation group. Skill increases significantly over time in the sham stimulation control group and is augmented further by each NEBS strategy. (**B**) Scatter plot of subcomponents of motor learning. All stimulation groups present significantly greater overall motor learning compared to the sham stimulation control group. Only unilateral anodal transcranial direct current stimulation (tDCS) reveals greater immediate effects on motor learning - *i.e.*, initial changes in skill after onset of stimulation, compared to sham control and transcranial random noise stimulation (tRNS). DC:M1-SO = unilateral tDCS. DC:M1-M1 = bilateral tDCS. RN:M1-SO = unilateral tRNS. *p<0.05, **p<0.01. Error bars = standard error of the mean. From Prichard *et al.* (2014)[6] with permission. Please click here to view a larger version of this figure.

Discussion

This protocol describes typical materials and procedural steps for modulation of hand motor function and skill learning using NEBS, specifically unilateral and bilateral M1 stimulation for anodal tDCS, and unilateral tRNS. Before choosing a particular NEBS protocol for a human motor system study, *e.g.*, in the context of motor learning, methodological aspects (safety, tolerability, blinding) as well as conceptual aspects (montage or current type specific effects on a particular brain region) need to be taken into account. Advantages and limitations of the three strategies are presented in **Table 1**.

NEBS type	Advantage	Limitation
Common to anodal tDCS and tRNS	Safe Cheap Easy to administer Outlasting effect on cortical excitability (up to 90 min) Improvement of motor function and motor skill learning in healthy subjects and patients with motor deficits Functional focality is reached by combination of NEBS with a particular task	Structural stimulation focality is limited and defined by electrode size and montage Larger electrodes may stimulate cortical areas adjacent to the M1 of interest
Unilateral M1 stimulation (tDCS)	Polarity specificity (direction of excitability change in M1 of interest can be chosen)	Receiving electrode (cathode) is an active electrode and may exert a confounding effect on underlying brain area Difficult participant blinding at higher stimulation intensities (current density > 40 μA/cm^2, *e.g.*, > 1 mA / 25 cm^2)
Bilateral M1 stimulation (tDCS)	Polarity specificity (direction of excitability change in M1 of interest can be chosen) Pronounced modulation of interhemispheric connection in addition to excitability increase of the M1 of interest (desired decreasing effect on the opposite M1)	Difficult participant blinding at higher stimulation intensities (current density > 40 μA/cm^2, *e.g.*, >1 mA / 25 cm^2) Higher risk of current shunting due to proximity of the electrodes
Unilateral M1 stimulation (tRNS)	Least side effects Improved participant blinding	No polarity-specificity Effects on excitability and motor behavior are more robust at high frequency spectrum (100-640 Hz)

NEBS, non-invasive electrical brain stimulation; M1, primary motor cortex; tDCS, transcranial direct current stimulation; tRNS, transcranial random noise stimulation

Table 1: Advantages and limitations of tDCS and tRNS.

From a methodological point of view subjects should always be screened thoroughly for contraindications for NEBS[3,41] using questionnaires or standardized interviews (*e.g.*, Keel *et al.*, 2001[25]). These do not differ between tDCS and tRNS. Absolute NEBS contraindications include: 1) skull deformation, *e.g.*, due to fracture, as it may influence current flow and promote unexpected side effects; 2) Implanted medical device, *e.g.*, cochlear implant and brain stimulator, as NEBS may negatively influence medical device functioning. For the use of TMS (*e.g.*, for motor cortex localization (see protocol step 2)) ferromagnetic objects in the head/neck area, (*e.g.*, shrapnel, surgical clips) also represent an absolute contraindication, as those objects may be dislocated by the magnetic field and pose a risk for the participant. Additional exclusion criteria are optional and depend on the study aims. Common additional contraindications include: 1) age above 85 years old; 2) pregnancy; 3) history of chronic skin disorders (mostly regarding the head); 4) adverse effects to previous brain stimulation protocols; 5) history of frequent or severe headache, *e.g.*, migraine; 6) history of epileptic seizures; and 7) pacemaker. For participants with pacemaker a minimum safety distance of 10 cm should be kept between stimulation site and the pacemaker to prevent interference with its functioning.

Subjects should not be stimulated if any of the absolute contraindications apply. For safety reasons the NEBS device should have maximum output in mA range, should be battery-driven and should not be used while the charger is connected to electrical outlet. When applied per protocol, tDCS and tRNS are usually well tolerated[32]. Side effects of stimulation may include itching, tingling sensation, and headache outlasting the stimulation duration or triggering migraine attacks. However, from estimated 16.000 tDCS sessions (including multiple sequential sessions) no severe tDCS side effects were reported (Bikson M., personal communication, 2015; meta-analysis in preparation). Side effects can be minimized by careful stimulation electrode preparation and placement. This includes: 1. Skin inspection for lesions, 2. Applying the stimulation via a conductive medium like rubber electrodes covered with conductive paste or with saline soaked sponges, 3. Fading in and out the stimulation (a longer duration of ramping up and ramping down (*e.g.*, 15 sec) is associated with less side effects), and 4. Impedance control. Participants usually habituate to skin sensations underneath the electrodes shortly after ramping up the stimulation. With tRNS in most cases skin sensations are less or not at all perceived compared to tDCS (consequently, similar rates of correct condition guess for sham and tRNS as compared to higher rates of correct condition guess with tDCS)[6]. This may be advantageous for studies where optimal blinding of participants is crucial. However, in the majority of studies participants were successfully blinded between real and sham tDCS, at least with low to medium stimulation intensities[32,42]. This is likely due to the implementation of a short ramping up and down for several seconds in the sham mode, which causes the tingling sensation[42] but apparently does not alter cortical function[2]. Using an "active" sham mode that elicits the tingling sensation and automatically turns off stimulation after some seconds may be a superior method for blinding both participant and researcher as compared to simply placing the electrodes on the head of the participant and not starting the NEBS device.

For comparability of publications indicate the current density, electrode size (*i.e.*, target area), electrode placement, conductive substrate between electrode and skin, duration for ramping up and down, stimulation duration and side effects. It should be noted that the declaration of stimulation intensity alone is not sufficient to estimate the current density delivered to the participant. For the calculation of current density divide the stimulation intensity (*e.g.*, 1 mA, 1.5 mA, 2 mA) by the stimulated area. For instance, if stimulation intensity is 1mA and the electrode size is 16 cm^2 the estimated current density is 0.0625 mA/cm^2 (*i.e.*, 1 mA/16 cm^2 or 62.5 μA/cm^2).

From a conceptual point of view, several cortical areas of the motor system are accessible by NEBS, either directly if the area is close to the cortical surface or via remote network effects[43,44]. The primary motor cortex can be located either by TMS-induced MEPs or using the EEG 10/20 international system[26]. Using the latter technique in a healthy participant is faster and easier as compared to using TMS-induced MEPs, but TMS provides superior accuracy to localize the individual cortical motor representation of interest. While the necessity for or the functional benefit from using a TMS hotspot as compared to the 10/20 system is yet unproven, TMS-induced MEPs demonstrate functional integrity of M1 and the pyramidal tract. For patients with brain lesion (*e.g.*, stroke) TMS-induced MEPs is therefore preferentially used to locate the motor cortical representation as it may be largely shifted due to lesion size and location, and secondary motor areas may generate the motor output.

NEBS electrode size or montage may impact cortical areas adjacent to the region of interest, resulting in limited focality of the stimulation itself[45,46]. Smaller electrode sizes (emitting electrode in case of tDCS) may limit the widespread and exert more focal effects onto M1, as suggested by modeling studies or software tools; similarly, less distance between electrodes may condense the electrical field[46,47]. However, the functional focality obtained by task specific activation of particular synapses[11] or networks that are augmented by combining task/training with stimulation could be more crucial[46]: on one hand, functional imaging studies revealed different network changes after unilateral versus bilateral M1 tDCS, or tDCS versus tRNS, respectively[14,15]. On the other hand, the net effect of anodal tDCS and tRNS on motor behavior, *e.g.*, learning, seems to be similar: Based on the few investigations with direct comparisons of stimulation type/montage, one could argue for positive effects on motor function as long as M1 contralateral to the tested hand is targeted by NEBS (in case of tDCS with anodal stimulation[4-6]).

Most robust behavioral effects are usually found when stimulation and task execution or training are carried out simultaneously[13]. Inconsistent results have been reported for NEBS and tasks applied consecutively[1]. Other electrode montages such as recently developed high-definition tDCS may increase stimulation focality[48,49] but require future investigation regarding the behavioral consequences. Controlled studies evaluating tRNS effects on stroke motor rehabilitation and learning, as well as comparative studies of distinct NEBS strategies in patient populations are largely missing. Future studies with NEBS of the human motor system are necessary for a better understanding of promises and pitfalls of NEBS in clinical applications.

Disclosures

The authors have nothing to disclose.

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