Targeting the frontoparietal network using bifocal transcranial alternating current stimulation during a motor sequence learning task in healthy older adults

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ARTICLE INFO

ABSTRACT

Background: Healthy older adults show a decrease in motor performance and motor learning capacity as well as in working memory (WM) performance. WM has been suggested to be involved in motor learning processes, such as sequence learning. Correlational evidence has shown the involvement of the frontoparietal network (FPN), a network underlying WM processes, in motor sequence learning. However, causal evidence is currently lacking. Non-invasive brain stimulation (NIBS) studies have focused so far predominantly on motor-related areas to enhance motor sequence learning while areas associated with more cognitive aspects of motor learning have not yet been addressed.
Hypothesis: In this study, we aim to provide causal evidence for the involvement of WM processes and the underlying FPN in the successful performance of a motor sequence learning task by using theta transcranial alternating current stimulation (tACS) targeting the FPN during a motor sequence learning task.
Methods: In a cohort of 20 healthy older adults, we applied bifocal tACS in the theta range to the FPN during a sequence learning task. With the use of a double-blind, cross-over design, we tested the efficacy of active compared to sham stimulation. Two versions of the motor task were used: one with high and one with low WM load, to explore the efficacy of stimulation on tasks differing in WM demand. Additionally, the effects of stimulation on WM performance were addressed using an N-back task. The tACS frequency was personalized by means of EEG measuring the individual theta peak frequency during the N-back task.
Results: The application of personalized theta tACS to the FPN improved performance during the motor sequence learning task with high WM load ($p < .001$), but not with low WM load. Active stimulation significantly improved both speed ($p < .001$), and accuracy ($p = .03$) during the task with high WM load. In addition, the stimulation paradigm improved performance on the N-back task for the 2-back task ($p = .013$), but not for 1-back and 3-back.
Conclusion: The performance during a motor sequence learning task can be enhanced by means of personalized bifocal theta tACS to the FPN when WM load is high, indicating that the efficacy of this stimulation paradigm is dependent on the cognitive demand during the learning task. These data provide further causal evidence for the critical involvement of WM processes and the FPN during the execution of a motor sequence learning task in healthy older. These findings open new exciting possibilities to counteract the age-related decline in motor performance, learning capacity and WM performance.

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1. Introduction

The ability to acquire new motor skills is important in daily life. Motor learning is a practice-dependent process in which movements are performed quicker and more accurately [1]. A vast amount of research has contributed to an increased understanding of the neural substrates and underlying mechanisms involved in the acquisition, consolidation and retention of new motor skills. Neuroscientific studies have focused predominantly on the motor network and the pivotal role of the primary motor cortex (M1) [2–4]. This is especially the case for non-invasive brain stimulation (NIBS) studies that attempt to improve motor learning by combining the practice of a challenging motor task with a stimulation paradigm [5–8]. However, studies have suggested that challenging motor tasks, such as motor sequence learning (MSL), do not rely exclusively on motor related processes, but also on cognitive processes, such as working memory (WM) [4,9,10]. Surprisingly, WM related brain areas have not been a target for NIBS paradigms intended to study MSL.

MSL is a process where independent movements are associated, eventually resulting into a multi-element sequence that can be performed quickly and accurately [4,11]. Studies have shown the involvement of WM in MSL [12–14]. WM refers to the ability to temporarily store and manipulate information in the mind [15]. Inter-individual variability in WM e.g., consists of the number of items that can be held and worked with [4]. This is important for MSL especially during the process of grouping elements of the sequence together in "chunks". This chunking process results into quicker execution of the movements [16–18]. Many studies have shown that healthy older show a decline in the ability to learn motor sequences [12,14]. Moreover, aging decreases cognitive functions including WM [19]. Therefore, an interaction among age, WM capacity and MSL has been recently suggested [14], though causal evidence in favour of this suggestion remains limited.

A promising neurotechnology to provide causal evidence is the use of NIBS, such as transcranial alternating current stimulation (tACS) [20–23]. This technique allows to exogenously interfere with ongoing oscillatory activity and to target specific networks, such as the fronto-parietal network (FPN), to enhance or decrease specifically respective cognitive functions, such as WM processes [24,25]. The FPN, a network related to WM, has shown to be activated during motor sequence tasks [18,26–29]. Cognitive processes rely on coordinated interactions within and among brain networks, implemented in the brain by oscillatory activity [30,31]. For example, efficiency is increased by oscillatory synchronization of neuronal firing, which creates ensembles of neurons that carry out specific computational functions [31,32]. The main working mechanism of tACS is to entrain or synchronize neuronal networks [20,33]. The stimulation frequency is adjusted to match the endogenous oscillatory frequency and its brain state. More specifically, tACS allows to exogenously interact with ongoing oscillations, which can result in enhanced coherence within networks with the respective behavioural impact [23,33,34]. Neuronal oscillations in the theta range (4–8 Hz) are engaged in WM tasks, with an increase in theta power during increased WM load [35–37]. Polania et al. and Violante et al. have shown a causal relationship between the synchronization of theta oscillations with a relative 0º phase difference in the FPN and the improvement of WM performance [24,25]. However, knowledge about the effects of tACS induced synchronization of theta oscillations in the FPN and MSL is lacking.

In this study, we aimed to determine a causal relationship between WM and MSL in healthy older adults. To do this, personalized theta tACS was applied to the right dorsolateral prefrontal cortex (DLPFC) and the posterior parietal cortex (PPC) intended to improve MSL by means of the training of the sequential finger tapping task (SFTT) [3]. To evaluate the importance of WM during MSL and how this is affected by the FPN stimulation, two versions of the SFTT were used. The versions differed in terms of low vs. high WM load. WM load was kept low by explicitly showing the sequence on a screen during the task [38]. In the high WM load version, the sequence had to be memorized prior to the task and was not shown during the task. This online maintenance of the sequence while performing the movements relies relevantly on WM processes [39]. In addition, we verify whether the present stimulation paradigm improves WM with the use of an N-back task [24]. With this study, we introduce the FPN as an additional stimulation target location for motor performance and learning enhancement and shine a light on the importance of taking cognitive processes into account during MSL paradigms.

2. Methods

2.1. Participants

In this study, we recruited N = 21 healthy, older, right-handed participants (N = 11 female, mean age ± sd: 69.6 ± 4.4, mean laterality quotient Edinburgh handedness inventory 85.03 ± 17.3) [40]. The data of N = 20 participants were considered due to a drop-out of one participant caused by an unrelated change in physical health. Inclusion criteria were: ≥ 60 years [41–43], right-handed and absence of contraindications for transcranial electrical stimulation (tES). Exclusion criteria were: neuropsychiatric diseases, history of seizures, medication that potentially interacts with tES, musculoskeletal dysfunction that impairs finger movements, professional musician, intake of narcotic drugs. All participants have signed an informed consent. The study was performed in accordance to the declaration of Helsinki [44]. Ethical approval was obtained from the cantonal ethics committee Vaud, Switzerland (project number: 2017-00765).

2.2. Experimental design

The design of this study was double blind, sham-controlled, cross-over. It consisted of two sessions before cross-over and two sessions after cross-over. During the session on day 1, the participants were informed, screened and asked to fill in three different questionnaires (tES safety questionnaire, Edinburgh Handedness Inventory (EHI), Center for Epidemiological Studies Depression Scale (CES-D)) [40,45]. Afterwards the participants performed an N-back test with EEG acquisition for peak frequency analysis. Following the EEG measurement, the participants did the motor training and the cognitive training with concurrent tACS. The next day the participants performed only the motor training with tACS. The stimulation condition was kept the same on both consecutive days and was changed after cross-over. The order of stimulation was defined in a pseudo-randomized fashion by an experimenter not involved in the data acquisition. The blindness for stimulation condition of both the participant and experimenter was ensured by an additional experimenter who set the parameters and turned on the stimulators during the experiment. Between the before and after cross-over sessions there was a minimum time period of two weeks, based on our previous work [46]. The same tasks, with different sequences, were repeated after cross-over, excluding the questionnaires. Please see Fig. 1 A for the timeline of study design.

2.3. Motor learning task

Participants executed two different versions of the SFTT based on the SFTT task used in earlier studies [3,47]. They were asked to

perform a 9-item sequence with their non-dominant left hand. The non-dominant hand was used to allow for a larger range of improvement [46]. They were orally instructed to continuously tap the same sequence as fast and as accurately as possible on a four-button keyboard (Current Designs, Philadelphia, PA, USA). The sequences consisted of 4 digits from 2 to 5, which corresponded to the four fingers from index (2) to the little finger (5) of the left hand. A cursor underneath the displayed sequence moved in response to every finger tap to identify the target digit, regardless of whether or not the button was pressed correctly. Different sequences were used for the baseline and the training measurements. All sequences were matched in complexity verified with the Kolmogorov complexity test [48]. The baseline measurement consisted of one block of 90 s, the training measurement consisted of seven blocks of 90 s, with 90 s breaks after every block which lasted 20 min in total. The task was implemented in Presentation software (Neurobehavioral Systems, Berkeley, CA, USA). Participants performed a low and a high WM load SFTT version. The low WM load version displayed the sequence on the screen asking participants to execute the sequence without prior familiarization. This version is referred to as the "non-memorized" version. For the high WM load version, participants had to memorize the sequence before the task started. They received the sequence on a paper and were asked to learn the sequence by heart, without practicing it on the button keyboard. With the use of a distractor task, during which they had to spell random words in a reversed order sufficient memorization of the sequence was verified. More precisely, participants had to spell backwards 3 words in a row, and recall the sequence out loud afterwards. After 3 times correct, the sequence was deemed sufficiently learned [49]. During the memorized version of the task, the participants could not see the sequence. Displayed on the screen

was a sequence of 9 "X's" with the moving cursor underneath to identify the target digit. Participants performed both versions divided over day 1 and day 2 in a randomized order. This order of versions was reversed after cross-over, see Fig. 1C.

2.4. Cognitive task

Participants were asked to perform the N-back task to verify whether this stimulation paradigm enhanced WM performance. The task was implemented in Matlab (The MathWorks Inc.,Natick, Massachusetts, USA) and was based on the single N-back task used by Jaeggi and colleagues [50]. The script was adapted from Quent, A.J [51]. in terms of language (French & English), length and difficulty level. The task consisted of a sequence of visual stimuli that were shown on a computer screen. The participants had to respond by clicking the right "Control" button on a computer keyboard when the stimulus was the same as the stimulus presented N positions back. Participants should not respond when a different stimulus was presented. The visual stimuli consisted of 10 random shapes, eight 8-points shapes (number 14, 15, 17, 18, 20, 22, 23, and 27) and two 12-points shapes (number 20 and 24) taken from Vanderplas and Garvin [52]. The stimuli were presented for 500 ms each with a 2500 ms interstimulus interval. The participants were required to respond within the response window that starts at the onset of the stimulus until the end of the interstimulus interval (3000 ms). The task consisted of 1 until 3-back levels, in that order. The task was divided into a baseline and training session, with the baseline session consisting of 1 block per n-back level (3 blocks in total) and the training session of 3 blocks per level (9 blocks in total). Every block consisted of 20 + n trials, with 6 targets and 14 + n non-targets. The reaction times, hits, misses, false alarms
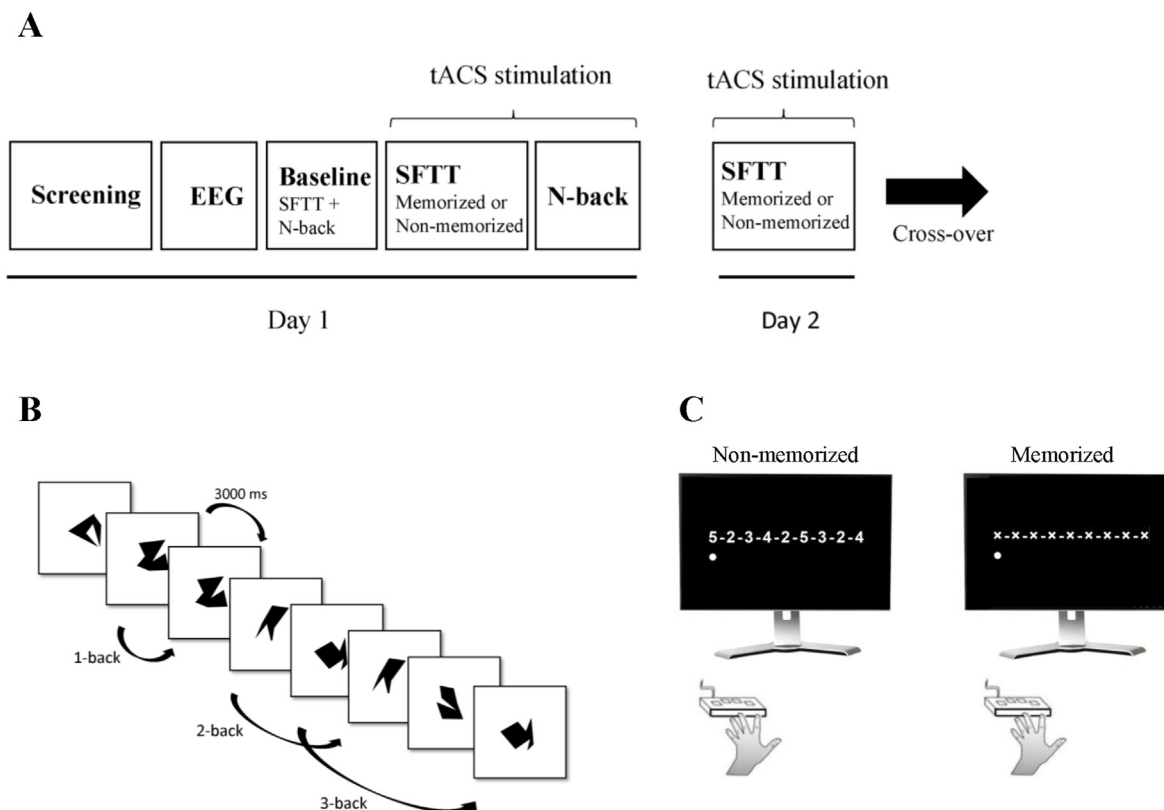

Fig. 1. Experimental design. **A)** timeline of study design. **B)** Example of N-back test with the three difficulty levels shown. **C)** Example of the non-memorized and the memorized version of the SFTT. Please note that with each key press advancing point indicates in both conditions just the position within the sequence.

and correct rejections were measured. Please see Fig. 1 B for a schematic illustration of the task. We had to exclude N = 9 before cross-over N-back task data sets due to an error in the response recording. A total of N = 31 N-back data sets were considered.

2.5. Transcranial alternating current stimulation

Multifocal tACS was applied to the right FPN using two neuro-Conn DC plus stimulators to enable bifocal stimulation (neuroConn GmbH, Ilmenau, Germany). Participants received both real (30 min) or sham (30 s) stimulation in randomized order, before or after cross-over [24,25,53]. The stimulation protocol consisted of the following parameters: in-phase (0º phase lag), intensity 2 mA (peak-to-peak) was gradually ramped up/down with an interval of 8 s. The in-phase stimulation between the two stimulators was assured by a repeated trigger from stimulator A to stimulator B after every completed cycle to signal the start of a new cycle [54]. The stimulation frequency was adjusted to the personal theta peak frequency, which was recorded during an EEG recording while performing a pre-baseline N-back test of 1 block per level. Rubber concentric electrodes were used: centre electrode size diameter: ca. 20 mm, area: ca. 3 cm^2 and ring electrode size diameter: out 100 mm/in 70 mm, area: ca. 40 cm^2. Electrode location was defined with the use of a standard 64 channel, EEG actiCAP with 10/20 system (Brain Products GmbH), targeting F4 corresponding to the dorsolateral prefrontal cortex (DLPFC) and P4 corresponding to the posterior parietal cortex (PPC). The paste used for conductivity with adequately low impedance was SAC2 electrode cream (Spes Medical Srl, Genova, Italy). This paste was adhesive which ensured stable electrode placements. The electrode placement and the electric field distribution were visualised with the use of standard template in SimNIBS (Version 3.2) [55]. The script to implement bifocal stimulation with ring electrodes was adapted from the open access Matlab script (© G. Saturnino, 2018). A template head model was used to simulate the electrode placement and electric field distribution. For the electrode placement and electric field distribution, please see Fig. 2 A & B. At the end of the last stimulation session, we investigated whether the stimulation was well tolerated and if there was a significant difference in experienced sensations between the real and sham condition. Moreover, we asked the participants to indicate whether they thought they had received real or sham stimulation during the before and after cross-over sessions. The stimulation sensations were described with the use of a structured interview [56]. We checked for the following

sensations: itching, pain, burning, metallic/iron taste in mouth, warmth, fatigue, other. With the possibility to respond: "none", "mild", "moderate", "strong".

2.6. EEG

All EEG recordings were done in a shielded faraday cage. A customized electrode set-up with 9 electrodes was used, Frontal (Fp1, Fp2, F3, Fz, F4), parietal (Cz, P3, Pz, P4), please see Fig. 2C. Using a 64-channel ANT Neuro EEG cap with eego^{tm}mylab software (ANT Neuro, Netherlands). EEG was recorded during the performance of the N-back task. With the use of markers, the beginning and the end of every separate N-back level were defined. Recordings were done during 3 N-back blocks resulting in approximately 3 min of recording time. The peak frequency in the theta range (4–8 Hz) was calculated using a custom Matlab script (The MathWorks Inc., USA) adapted from the script used by Salamanca-Giron and colleagues [54] and made suitable for theta frequency analysis during N-back task performance. Theta frequencies for tACS were personalized similar to previous work [54,57]. However, we did not intend to compare the efficacy of tACS with personalized frequencies to tACS with standard (non-personalized) frequencies. Therefore, this study does not aim to demonstrate beneficial physiological effects of tACS with personalized over tACS with standard (non-personalized) frequencies. The target electrodes F4 & P4, which are the same as the stimulation locations show small variance in recorded theta frequency. The average theta frequency for the F4 electrode was 4.71 (range 4.12–7.77) and for the P4 electrode 4.97 (range 4.11–6.84).

2.7. Data analyses

Normality of the data was visually checked with histograms and Q-Q plots of residual values and confirmed by verification of skewness ranging between 1 and -1 [58]. P-values of < .05 indicate statistical significance. Pre-processing of the behavioural data of the SFTT was done with an in-house script implemented in Matlab. Main output measures were: correct sequences, total completed sequences and correct sequences/completed sequences. Preprocessing of the individual N-back data was done with RStudio (version 1.4.1717, 2021) [59]. Individual data were combined in one main file using Microsoft Excel. For analysis, the data was normalized by subtraction to the baseline block related to the stimulation condition. Normalization was performed in the view of
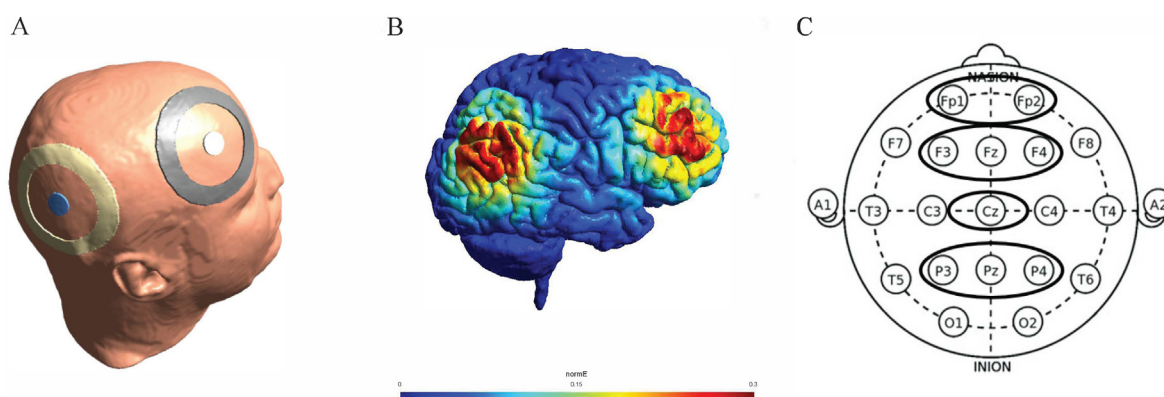

Fig. 2. Bifocal tACS application and EEG recording. **A**) Bifocal electrode placement for tACS with concentric electrodes placed on F4 and P4. Image created with the use of SimNIBS software. Head is derived from a standard template provided. **B**) Simulation of the electric field distribution of tACS set-up created with the SimNIBS software. Label indicates strength of electric field (V/m). Brain is derived from a standard template provided in the program. Stimulation parameters are adjusted to the current study. **C**) EEG recording sites for the determination of the individual peak frequency during the working memory task.

heterogenous performance levels, especially typically found in older subjects to provide better comparability between subjects. The baseline blocks were compared in R using paired-samples t-tests. The equality of the baseline blocks was verified using Bayesian statistics by computing a Bayesian paired-samples t-test with the use of JASP software (version 0.16.0.0). All other analysis of the SFTT and the N-back data were done in Rstudio (version 1.4.1717). Data were analysed with the use of Linear mixed-effects models that were fitted with the "lmerTest" package. Output was type III anova table with p-values for F-tests [60]. Effect size was determined using partial eta squared with the "effectsize" package. Post-hoc analysis was done by pairwise comparisons, using the estimated marginal means and Tukey correction. Analysis of the tACS stimulation sensations and blinding responses were analysed with JASP (version 0.8.5.1) [61]. Responses to the real vs. sham stimulation estimations were analysed using a binomial test. The stimulation sensations were analysed using contingency tables with chi-squared analysis to control for differences between the real and sham stimulation conditions.

3. Results

3.1. Sequential finger tapping task

The two SFTT's have been analysed separately as they differ in amount of WM-load (high and low WM load). Prior to the main analysis, the baseline performance between active and sham stimulation was compared and was not significantly different for both the memorized condition $t(19) = 0.72$, $p = .48$, $d = 0.16$, and the non-memorized condition $t(19) = 0$, $p = 1$, $d = 0$. To further analyse the null-result and to confirm equality of the groups active vs sham groups were compared in both conditions using Bayesian statistics. The analysis indicated for the memorized condition $BF_{01} = 3.41$, meaning it is 3.4 times more likely that the baseline results are equal than different. The non-memorized condition indicated $BF_{01} = 4.3$, therefore is it 4.3 times more likely that the baseline groups are equal.

In this study, online learning is defined as a significant improvement of behavior within the training session. A significant effect of stimulation on learning is defined by a change in improvement dynamics during the training. With the use of a linear mixed effects model the analysis of the amount of correct sequences of the memorized version of the SFTT showed a significant effect for blocks $F(6, 247) = 18.57$, $p < .001$, $\eta_p^2 = 0.31$ indicating a large effect size, as well as a significant effect for stimulation $F(1, 247) = 18.83$, $p < .001$, $\eta_p^2 = 0.07$ with a medium effect size, but no blocks × stimulation interaction $F(6, 247) = 0.77$, $p = .59$, $\eta_p^2 = 0.02$. To further define the effect of stimulation on learning, we determined the difference between the conditions at the end of the training, which showed a strong trend for a significant difference $t(19) = -2.07$, $p = .052$, $d = -0.46$. The results of the non-memorized version show a significant effect for blocks $F(6, 247) = 16.00$, $p < .001$, $\eta_p^2 = 0.28$ (large effect), but no stimulation $F(1, 247) = 0.46$, $p = .499$, $\eta_p^2 = 0.002$ or interaction effect $F(6, 247) = 0.36$, $p = .901$, $\eta_p^2 = 0.009$. Indicating that in both conditions, participants learned significantly, but only in the memorized condition there was a significant effect of tACS stimulation on performance, see Fig. 3. The lack of an interaction effect does not allow to conclude a significant effect of stimulation on motor learning although the trend for a difference in performance on block 7 indicates a potential for stimulation effect. To further investigate the results on the SFTT and appreciate the variance in performance the individual trajectories of the participants are indicated in the

supplementary material, Supplementary Fig. 1. Analyses with non-normalized data revealed comparable findings, for details please see the supplemental online material (SOM) and SOM Fig. 3.

3.2. Speed and accuracy

To further investigate the results of the **memorized** condition, the total amount of completed sequences were analysed as a measure of speed. The results showed a significant block effect $F(6, 247) = 28.21$, $p < .001$, $\eta_p^2 = 0.41$ (large effect) and a significant stimulation effect $F(1, 247) = 15.92$, $p < .001$, $\eta_p^2 = 0.06$ (small effect), but no interaction effect $F(6, 247) = 0.23$, $p = .968$, $\eta_p^2 = 0.006$, see Fig. 4A. Although the active stimulation group is faster compared to the sham group, the similar pattern of improvement points towards a performance rather than a learning effect.

In order to see whether the increased amount of correct sequences was driven by faster sequence execution or by a simultaneous increase of accuracy, we analysed the ratio between the total amount of sequences and the correct sequences as an accuracy measure. Upon inspection, the real stimulation group shows different dynamics in accuracy than the sham group. The real stimulation group demonstrates a steep significant increase in accuracy between the first and the second training block while the sham group's increase is more gradual $t(19) = -2.68$, $p = .015$. The accuracy between the groups during the 1st training block was not significantly different $T(19) = 0.85$, $p = .404$. Therefore, to visualize the difference in dynamics we measured the difference in accuracy with regard to block 1. Results showed a significant block effect $F(6, 247) = 3.47$, $p = .003$, $\eta_p^2 = 0.08$ (medium effect), and a significant stimulation effect $F(1, 247) = 18.31$, $p < .001$, $\eta_p^2 = 0.07$ (medium effect), but no block × stimulation interaction $F(6, 247) = 0.85$, $p = .529$, $\eta_p^2 = 0.02$, see Fig. 4B. In an additional analysis the comparison of behavior on block 7 shows a significant difference between verum and sham $t(19) = -2.31$, $p = .032$, $d = -0.51$. Therefore, although the lack of an interaction effect does not signify significant motor learning effects, the results do indicate that accuracy significantly improved with stimulation in the early stage of training, which remained significantly different in the last block. Analyses with non-normalized data revealed comparable findings, for details please see SOM and SOM Fig. 4.

3.3. N-back task

The N-back task performance was analysed by the following outcomes: hits, false alarms, accuracy (hits − false alarms), and reaction time for hits. All parameters were analysed separately using linear mixed effects models. The stimulation conditions (real vs. sham) and the three N-back difficulty levels were included as independent variables in the model. Two separate analyses were performed, one model included difficulty levels 1 and 2 to mimic the conditions comparable to the study of Violante et al. (2017), additionally we added difficulty level 3 to the model to test for a stimulation effect on the task with higher cognitive demand [24].

3.4. Reaction time

We were able to replicate the results of Violante and colleagues for the parameter reaction time with a significant effect of stimulation $F(1, 47.10) = 5.33$, $p = .025$, $\eta_p^2 = 0.1$ (medium effect), as well as an effect for difficulty level $F(1, 35.77) = 44.61$, $p < .001$ $\eta_p^2 = 0.55$ (large effect), and an interaction effect $F(1, 34.54) = 4.83$, $p = .035$, $\eta_p^2 = 0.12$ (medium effect). Post-hoc analysis with Tukey correction showed a significant difference between sham and real stimulation
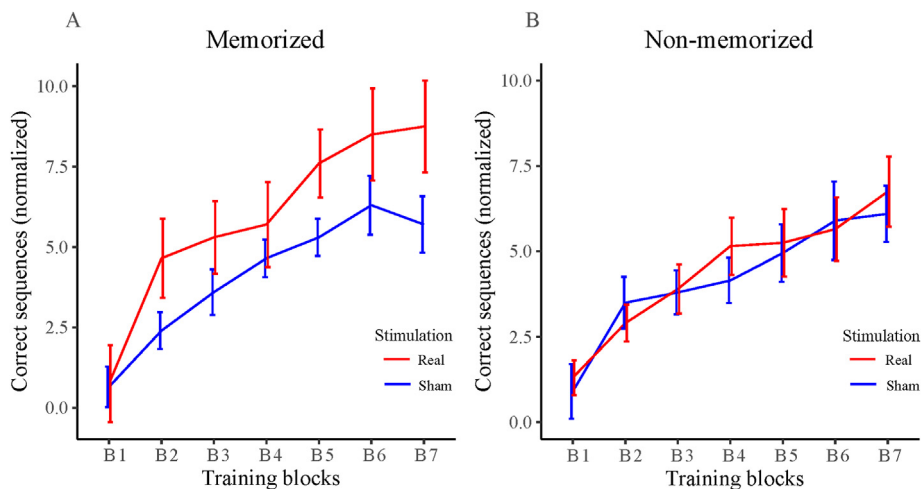
Fig. 3. Plot of the correct sequences of the SFTT, results are normalized to baseline by subtraction. Error bars show standard error of the mean (SEM). On the left, the results of the memorized version. On the right, the results of the non-memorized version. Red lines depict the real stimulation and blue lines are the sham stimulation. Please note a significant stimulation effect with enhanced behavioural improvement in the memorized version (left graph). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

during difficulty level 2, t(42.1) = 3.22, p = .013, but not for level 1 t(42.6) = 0.20, p = .997. Both conditions showed a significant increase in reaction time between level 1 and level 2, which was more prominent in the sham condition t(36.4) = −6.16, p < .001 than the real condition t(36.4) = −3.25, p = .013 Adding the 3-back difficulty level to the model resulted in no effect for stimulation F(1,78.24) = 1.61, p = .209, a significant effect for difficulty level F(2, 67.65) = 34.99, p < .001 and no interaction effect F(2, 67.65) = 0.75, p = .478, see Fig. 5.

3.5. N-back performance parameters (hits, false alarms, accuracy)

The analyses did not show a main effect of stimulation on any of these parameters for the 2 and the 3 level of difficulty models. There was a significant main effect of difficulty level. Indicating a significant decrease in performance with increasing n-back levels on all parameters. There were no stimulation × difficulty interaction effects. Please see Table 1 for statistical results.

3.6. Peak frequency analysis

During the EEG measurements data from 9 electrodes were acquired during the performance of the pre-baseline measurement of the N-back task. The individual average peak frequency of the 3 N-back levels combined was used as the personalized theta stimulation frequency for the rest of the study. Results of the overall average peak frequency showed a group mean of 4.5 (sd = 0.28) with a range between 4.1 and 5.4. For more details, please see Table 2.

3.7. Stimulation sensations & blinding

Based on the stimulation sensation interview, there were no adverse effects due to the tACS stimulation and only minor tACS sensations were reported. Most participants responded either with "none" or "mild". Moreover, there was no significant difference between the stimulation and sham condition for any of the perceived sensations, see Table 3.
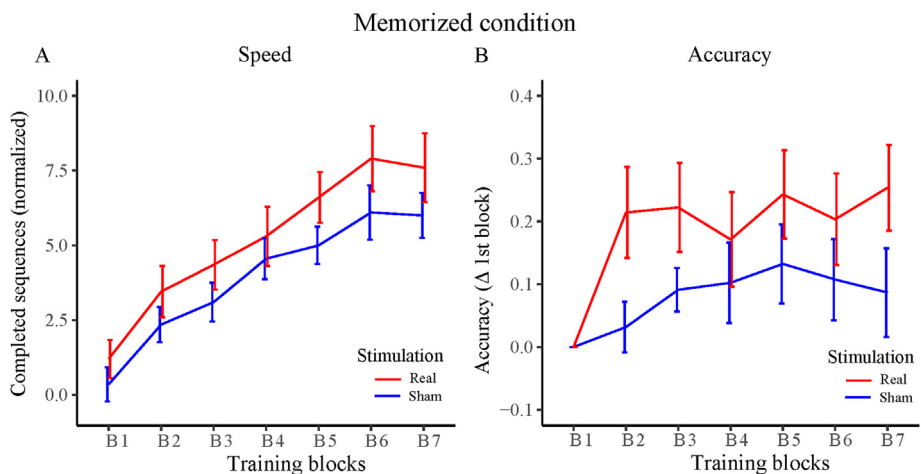

Fig. 4. Plots of the speed and accuracy of the SFTT in the memorized condition. The error bars indicate standard error of the mean (SEM). Plot **A)** shows the speed determined by the total amount of completed sequences. Higher numbers depict better performance. Plot **B)** Shows the difference in accuracy as determined by the ratio of correct sequences divided by completed sequences, with regard to the first block. Please note the tACS significantly enhanced both, speed (A) and accuracy (B).
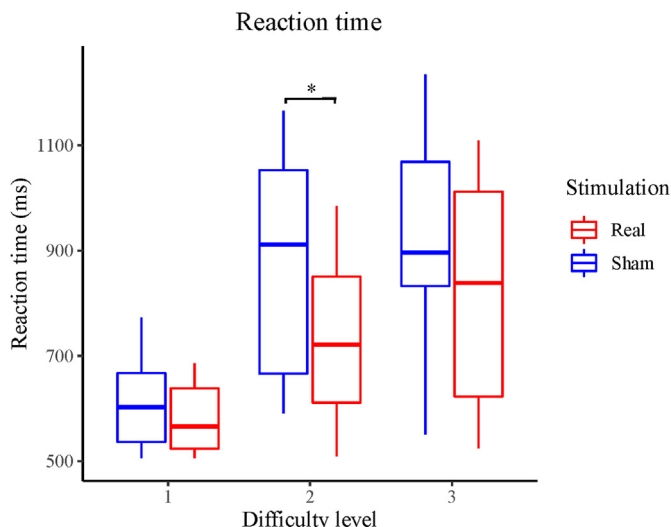
Fig. 5. Figure of the reaction time for the hit responses on the N-back task. Difficulty levels 1-, 2- and 3-back included. The box ranges from Q1 (the 25th percentile) to Q3 (the 75th percentile), the bar shows the median. Results show better performance in reaction time during level 2-back for the real stimulation condition compared to sham.

Participants were not able to discriminate between real and sham stimulation. A binomial test indicated that the proportion of correct answers during session 1 was 0.4, which was not significantly different than the chance level (0.5), $p = .503$. For session 2, the proportion of correct answers was 0.6, which was not significantly different than chance, $p = .503$, see Table 4.

4. Discussion

The main outcome of this study is that personalized, bifocal, synchronized tACS to the right FPN can enhance the performance during a SFTT with high WM load in healthy older. In contrast, this interventional paradigm did not affect the performance during the SFTT, if the WM load was low. These findings indicate that the efficacy of bifocal theta tACS applied synchronously to DLPFC and PPC is dependent on the cognitive requirements and underlying cognitive state during the task. This aspect is further supported by the findings that tACS also improved N-back task performance specific to difficulty levels that were demanding enough.

4.1. Motor task

The present results support the view of a causal effect of synchronized bifocal theta frequency oscillations applied to the right FPN on the performance of a motor sequence learning task.

Table 2
Peak frequency analysis.

	Mean Theta	1-back	2-back	3-back
Mean (sd)	4.48 (0.28)	4.42 (0.35)	4.46 (0.46)	4.51 (0.65)
Minimum	4.1	4	4	4
Maximum	5.4	5.7	6	7.8
Missing	0	0	0	1

Descriptive statistics of the peak frequency analysis measured during the three levels of the pre-baseline N-back measurements. The results show the overall means from the three levels combined and the means per N-back level. For the personalized stimulation paradigm, the individual mean peak frequency of the 3 N-back levels combined was used.

Correlative evidence of the activation of the FPN during finger tapping tasks has been previously shown using neuroimaging [62,63]. The meta-analysis of Witt and colleagues (2008) has shown that visually or self-paced finger tapping tasks induce concordant activity in the right DLPFC and the right inferior parietal cortex [63]. However, to the best of our knowledge, this was the first time the FPN was used as a target for a tACS paradigm with the intend to improve MSL.

Here, we were able to demonstrate an improvement in performance and a hint to a possible improvement of the training effects with this approach, but exclusively for the SFTT condition with high WM load (memorized condition). Therefore, the efficacy of the present orchestrated stimulation paradigm on motor behaviour was dependent on the amount of WM load during the task. This is in line with the study of Violante and colleagues (2017) that showed that theta tACS to the right FPN improved performance on a WM task, but only for the task with higher WM load [24]. This might be explained by the fact that the FPN shows more coherence in the theta range during WM tasks with high WM load [64]. With the use of tACS it is suggested to be able to exogenously enhance coherence by the entrainment of the cortical oscillation between distant regions [20]. Although we did not verify network coherence with the use of EEG or other neuroimaging measures, we hypothesize that exogenously induced theta oscillations might have amplified the ongoing physiological oscillatory activity engaged in WM processing, which in turn has supported the performance and acquisition process of the motor task with high WM load, but not with low WM load.

Another possible explanation is that the involvement of the FPN is related to a specific sub-process of WM. WM can be roughly divided into three sub-processes: encoding, maintenance and retrieval [65]. The non-memorized SFTT condition required the participants to learn the sequence while performing the movements, which falls under the encoding phase. During the memorized SFTT condition, the participants needed to maintain and retrieve the previously learned sequence while performing the

Table 1
N-back parameters.

Parameter	Statistics		
Model 1	Stimulation	Difficulty	Interaction
Hits	$F(1, 40.4) = 0.80$, $p = .375$	$F(1, 34.5) = 39.65$, $p < .001$	$F(1, 33) = 0.49$, $p = .488$
False alarms	$F(1, 33.1) = 0.54$, $p = .467$	$F(1, 29.4) = 56.78$, $p < .001$	$F(1, 29.4) = 0.87$, $p = .357$
Accuracy	$F(1, 32.1) = 0.12$, $p = .733$	$F(1, 25.9) = 85.12$, $p < .001$	$F(1, 25.9) = 0.05$, $p = .823$
Model 2	**Stimulation**	**Difficulty**	**Interaction**
Hits	$F(1, 75.4) = 0.70$, $p = .407$	$F(2, 65.2) = 69.46$, $p < .001$	$F(2, 65.2) = 2.05$, $p = .137$
False alarms	$F(1, 76) = 1.05$, $p = .309$	$F(2, 65.8) = 42.26$, $p < .001$	$F(2, 65.8) = 0.43$, $p = .651$
Accuracy	$F(1, 75.5) = 0.02$, $p = .892$	$F(2, 65.4) = 65.38$, $p < .001$	$F(2, 65.4) = 1.57$, $p = .215$

Statistical results of all the n-back parameters. Columns show the main effect and the interaction effect of the independent variables. Model 1 shows the analysis with difficulty level 1 and 2, model 2 additionally includes level 3.

Table 3
Stimulation sensations.

	None		Mild		Moderate		Strong		Statistics	
	Real	Sham	Real	Sham	Real	Sham	Real	Sham	Chi-square	p-value
Itching	75	80	20	15	5	5	0	0	0.18	.916
Pain	85	85	5	15	10	0	0	0	3.00	.223
Burning	80	80	10	20	10	0	0	0	2.67	.264
Warmth	70	90	30	10	0	0	0	0	2.50	.114
Metallic/iron taste	100	100	0	0	0	0	0	0	n/a	n/a
Fatigue	85	80	15	20	0	0	0	0	0.17	.677
Other	80	80	15	15	5	5	0	0	0.00	1.000

tACS sensations shown in percentages of participants who chose that specific response option for the intensity of the sensation. Statistics show comparison between real and sham stimulation.

movements. A recent meta-analysis has shown that during the transition from encoding to maintenance and retrieval stages, the involvement of the FPN progressively increases. Therefore, it can be well hypothesized that the memorized SFTT condition benefits more from the FPN as a target while the non-memorized SFTT condition profits more from stimulation of other brain regions. For instance, the acquisition phase relies heavily on the dorsal attention network, which predominantly includes the frontal eye fields and the intraparietal sulci [65,66]. Moreover, studies have shown a high involvement of the M1 during the early stages of learning, with a reduction of activity to baseline when a sequence becomes explicitly known [67,68].

The involvement of the frontal and parietal areas during MSL has been well established, however their precise functional role is less clear [67,69–71]. MSL can be divided into three different learning phases: stage 1 for acquisition, stage 2 for consolidation and stage 3 for retention. The early learning phase relies more heavily on cognitive processes such as WM, showing an activation in the prefrontal cortex and parietal areas [72–74]. In this study, the efficacy of targeting the FPN to enhance performance on the MSL task is most likely specific to WM load. Studies that focused on the WM processes found that the FPN is associated with the maintenance and manipulation of information when theta oscillations were in synchrony between the two brain areas [25,64]. This might explain why the performance on the motor sequence task only improved during the high WM load task, where the participants had to perform the sequence from memory. Moreover, both accuracy and speed improved significantly in the memorized condition due to the tACS stimulation. However, the real stimulation induced a sharp increase in accuracy, while the sham group improved more gradually. Similar results have been shown in a study comparing real vs. sham anodal transcranial direct current stimulation (atDCS) applied to the M1 on a SFTT. Different age groups were compared and older adults showed a sharp increase in accuracy in the real stimulation group and a gradual increase in the sham group [75]. They argue that the active M1 stimulation facilitated the encoding

Table 4
Descriptive statistics for the tACS blinding.

Session	Answer	Frequency	%
Before cross-over			
	Correct	8	40
	Incorrect	12	60
	Total	20	100
After cross-over			
	Correct	12	60
	Incorrect	8	40
	Total	20	100

The frequencies of correct and incorrect distinctions between real and sham stimulation before and after cross-over.

and storage of the sequence in memory. In the current study, the stimulation target was the FPN and was effective in the memorized condition when the sequences were already learned. This result could be driven by an enhanced capacity to maintain and retrieve the previously learned sequence, due to the synchronization of theta oscillations in the FPN [65].

This study aimed to extend previous studies that have targeted the FPN with bifocal theta tACS to improve WM performance [24,25,76,77] by using a similar setup to study the effect on MSL. This has been the first time that both the DLPFC and the PPC have been targeted with the use of bifocal theta tACS during a motor sequence learning task. The main aim was to target the FPN as a network that has shown to be important for WM and has shown activation during MSL [18,24–29,76,77]. Although we were able to show that bifocal tACS to the FPN was effective when WM-load was high, we cannot exclude that this effect might have been generated by a monofocal stimulation of either the DLPFC or the PPC. This study did not intend to compare the efficacy of monofocal theta tACS to bifocal theta tACS on MSL. However, based on the positive effects of targeting these areas with bifocal theta tACS on the performance of a MSL task more research is necessary to define the exact working mechanisms and to determine the effects of monofocal stimulation to either of the two areas separately. Due to the lack of comparative studies, no final conclusive statement can be made about the beneficial effects of bifocal FPN stimulation over targeting one single of the target brain areas. Further research in upcoming studies will have to address this open question in detail.

4.2. Personalized tACS

This study has used personalized tACS stimulation in the theta range on MSL and cognitive function. This approach was based on a study of Reinhart and Nguyen (2019) who showed beneficial effects of personalized fronto-temporal theta tACS compared to a standard theta tACS on a WM task in healthy older adults [57]. Individual peak frequencies were measured while participants performed the N-back task to determine the individual stimulation frequency, though a comparison between personalized and standard theta was not in the scope of the present study. We assume that individualizing stimulation paradigms might be important due to a more effective peak frequency as suggested by e.g., Reinhart and Nguyen, but also based on the differential functional effects of low theta frequencies (4–4.5 Hz) compared to high theta frequencies (7 Hz) on WM performance [78–80]. More specifically, 4 Hz tACS to the right parietal cortex improved WM capacity, while 7 Hz tACS reduced WM capacity in healthy young adults [78–80]. Jones et al. compared bifocal 7 Hz tACS to 4.5 Hz tACS applied to the FPN and found positive effects for 4.5 Hz, but not 7 Hz stimulation on WM performance [79]. However, there are also reports, which did not show effects of personalization such as in a current TMS study [81].

The average stimulation frequency in the present study was 4.5 Hz, which fits with the abovementioned low theta frequencies relevant for WM. As the comparison between personalized and standard (non-personalized) theta tACS was not in the scope of the present study, we cannot draw any conclusion whether personalization in the present study is more effective than non-personalized bifocal tACS in the theta range, an interesting question that has to be addressed in upcoming studies.

4.3. N-back task

The reason for the use of the N-back task was twofold. First and foremost, as a way to measure the individual theta frequency while performing a WM task. Second, it was used as an additional control experiment to verify that the stimulation was indeed directed to the FPN and modulates a key function processed by the FPN. The behavioural results of the WM task support the notion that theta tACS to the FPN enhances WM performance [24,25]. As there was no neuroimaging data to confirm that the FPN was indeed targeted, a behavioural difference in WM performance provides correlational evidence.

In this study, we could replicate the observations of Violante et al. showing that exogenous synchronization of cortical oscillations in the theta range improved WM performance when cognitive demands were moderately high (2-back level) [24]. We have extended the results with showing that this was only applicable to level 2-back and not the more difficult 3-back level. The efficacy of the stimulation paradigm seems to follow an inverted u-shape in relationship to the difficulty of the task. The present study cohort were healthy older adults. Although it is currently unclear whether young adults would still benefit from the oscillatory synchronization during the 3-back task, one could speculate that the inverted u-shape with the peak at the 2-back task is age related.

Studies that have compared performance on WM tasks between young and healthy older adults have shown age related reduction in performance especially in tasks with high cognitive demand [82,83]. In response to high WM load, older adults show a relative hypoactivation in fronto-parietal regions compared to young adults [82,84]. The "Compensation-Related Utilization of Neural Circuits Hypothesis" provides a framework for this phenomenon; age-related hyperactivations are seen during tasks with low WM load due to reduced neural efficiency, with hypoactivation for tasks with high WM load due to reduced neural capacity [85]. Showing that older adults use compensatory mechanisms already with low WM load tasks (1-back) and are therefore not able to recruit the necessary neural resources during high WM load tasks (3-back) [82,83]. Heinzel and colleagues hypothesized that the change in neuronal activity is due to a decrease in FPN coupling; they showed that fronto-parietal connectivity decreased in older adults during 2-back and even more during 3-back tasks [73,86]. This could indicate that the difference in efficacy of stimulation between the 2-back and the 3-back tasks is related to the degree of deficient coupling of the FPN within these tasks and that the interventional approach with tACS could only sufficiently compensate these mechanisms for the 2-back task, but not any more for the 3-back task. The lack of improvement during the 1-back condition could indicate that the natural compensatory mechanisms are not sensitive to the effects of this stimulation paradigm. This points towards a specific efficacy that is dependent on the brain state caused by the amount of WM load.

The results of the N-back task showed a specific effect on reaction times, and not on hit-rate, false alarms, and accuracy. These findings are similar to previous studies by Polania et al. (2012), Violante et al. (2017), and Alekseichuk et al. (2017) that used theta tACS to target the FPN. Synchronized tACS decreased reaction times

[24,25], while desynchronized tACS increased the reaction time on a visual WM task [25,76]. The exact reason for the effect on reaction times but not on other parameters remains elusive. Violante et al. showed a relation between increased parietal BOLD activation and decreased reaction times [24]. Evidence suggests a critical role of the parietal area in WM maintenance [87]. Therefore, Violante et al. suggest that the increase in neural activation in the parietal areas might have interacted with the mechanisms related to reaction times [24]. However, Alekseichuk et al. argue that the improved reaction times are network-related, as they found increased reaction times after desynchronization of the prefrontal areas from the parietal areas [76]. They argue that this is due to a decline of information uptake, reflected in the outlasting theta rhythm desynchronization in the cortex [76]. Although the results seem to point towards specific effects of synchronized theta tACS on reaction times, the exact mechanisms remain unclear. Further analysis is necessary to disentangle the exact physiological mechanisms of responses during WM tasks.

4.4. Future steps

The present study was a proof-of-principle study with the aim to investigate the involvement of the FPN in motor sequence learning. This study has a few limitations, which are discussed by means of suggestions for future studies. Firstly, the present data suggest a clear effect on behavioural performance of the interventional approach during the task, however whether it really impacts on learning is not clear. There was no clear statistical interaction between condition and blocks that would substantiate a strong learning effect, though there are probably hints towards a potential additional effect on learning by means of changes in the course of accuracy and the trend to a difference for total learning at the end of the training. This important open question has to be addressed in detail in upcoming studies with more intensive training (e.g., longer training session, multiple training sessions). Moreover, follow-up sessions will enhance our understanding about the consolidation and possible retention of behavioural improvement. Motor learning encompasses multiple processes such as online and offline learning. Online learning is the improvement during the training of the task; offline learning happens after training and is a vital part of the consolidation of learned behaviour [67,88–90]. Multiple sessions will allow to investigate whether improvement continues with multiple training sessions, impacts differentially on on-line and off-line learning (or only on performance) and whether it retains during longer periods. Secondly, we currently cannot conclude whether personalizing of the stimulation frequency is beneficial compared to a standardized frequency (e.g., 6 Hz) for stimulation in the present study [23–25]. This aspect was beyond the scope of the present study and has to be addressed in upcoming studies. Comparing the standardized to a personalized stimulation paradigm will than provide more conclusive results about the importance of personalization to endogenous oscillatory activity within the present task. Lastly, to further personalize the approach future studies should personalize the placement of electrodes to the individual brain based on simulations. In the current study the electrode placement was defined by standardized locations using an EEG cap with the 10/20 system. We have used concentric electrodes and each montage consisted of a small circular centre electrode surrounded by a larger return electrode. This set-up has shown to improve focality compared with other electrodes such as the 5×5 cm rectangular electrodes or ring electrode set-ups with the return electrode on a separate region [91]. For a simulation of the electric field distribution, please see Fig. 2B. This improved focality highlights the importance of precision of the electrode placement as the stimulation is most

effective close to the centre of the electrodes [92]. The currently used technique based on the 10-20-electrode system has been widely used in NIBS studies [93]. However, this is a standardized electrode placement system based on anatomical landmarks that can vary across participants [94]. A recent study of Scrivener and Reader compared the locations of the electrode placements with the use of an EEG cap with MRI images of the same participants. They found that the electrode placements deviated from the actual cortical locations with the smallest SD of 4.35 mm in frontal areas and the largest SD of 6.25 mm in the occipital and parietal areas [95]. These deviations are unlikely to result in any behavioural differences due to the focality of the stimulation. However, it does show that there is room for improvement in terms of precise definition of target locations and consistency in electrode place-ment. A way to improve precision is by using neuronavigation techniques guided by structural neuroimaging or with the use of functional MRI to pinpoint the exact target locations for stimulation [93].

5. Conclusion

In conclusion, in this study, we were able to show a causal relationship between stimulating the FPN and improvements on a MSL task. Moreover, we were able to show distinctive efficacy of FPN synchronization for motor tasks with low- and high WM load, resulting in improved performance on the motor task with high WM load, but no stimulation effects on the motor task with low WM load. Despite the clear effect on the performance level there was no clear effect, probably a hint, towards enhancement on motor learning, an aspect that have to be addressed in detail in upcoming studies. The mechanisms of action point towards an ef-fect of the stimulation on an improved capacity to maintain and perform the sequences. The current knowledge about using tACS to target frontal and parietal areas to improve MSL is limited. How-ever, these results indicate that targeting the FPN as a network using personalized bifocal oscillatory stimulation is a promising approach. In addition, the present study showed that theta tACS applied to the FPN improved WM performance. This reveals an important interplay between the motor and cognitive domain pointing to it as a promising target for interventional strategies based on NIBS. However, to do this successfully, it is critically important that such an approach might only be effective when then cognitive load of a respective task is significantly high as demon-strated here by the WM load.

Taken together, personalized orchestrated bifocal tACS applied to the FPN improved performance on a MSL task. This might offer a promising strategy to enhance motor skills and motor learning in healthy older adults and neurological patients showing deficits in motor performance and/or motor learning.

Funding

CRediT authorship contribution statement

L.R. Draaisma: Conceptualization, Design of experiment, Methodology, Validation, Data acquisition, Formal analysis, Writing — original draft, Writing — review & editing, Visualization, Project administration. **M.J. Wessel:** Conceptualization, Project adminis-tration, Writing — review & editing. **M. Moyne:** Validation, Randomization, Data acquisition. **T. Morishita:** Writing — review &

editing. **F.C. Hummel:** Conceptualization, Design of experiment, Interpretation of results, Writing — review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2022.06.012.

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