Master Thesis in Human Factors and Engineering Psychology

The use of TBS to investigate the involvement of SMAproper and preSMA in a motor-sequencing task

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Abstract:

In the present study the use of Theta Burst stimulation (TBS) was evaluated and two design protocols by Verwey et al. (2002) and Ruitenberg et al. (2014) are combined to one experimental design to investigate the functionalities of the SMAproper and preSMA in one single experiment. A between-subjects design was used to compare the performance of participants on a discrete sequence production (DSP) task. TBS was applied to stimulate either the preSMA or SMAproper to inhibit their functions and study the effects on motor-sequencing performance. Based on the two previous studies by Verwey et al. (2002) and Ruitenberg et al. (2014), it was expected that stimulation of the preSMA would slow down motor chunk initiation and stimulation of the SMAproper would slow down overall reaction times (RTs). The findings of the previous studies could not be replicated in this study. This is perhaps because of the use of TBS instead of rTMS. It is concluded that the use of TBS was not sufficient to inhibit the activity of the preSMA and the SMAproper and therefore had no influence on RTs and chunk initiation. Intensive literature research was done to explain the absence of any effects. For example, muscle contraction before, during and after the stimulation and the learning effect that was maybe too strong were possible explanation. Therefore, a follow-up study with some adjustments regarding the experimental setup is suggested for future research.

Table of contents

1. Introduction

Daily activities, such as riding a bike, lacing a shoe or shifting gears, are an indication for the human ability to develop a motor skill. In the field of cognitive psychology ample research is done aimed to understand the development of a motor skill and how the cognitive system is able to control the motor system. Most of the complex motor actions that people perform in their daily lives consist of series of simple movements that are executed in a specific order. With practice, the execution and the order of these movements is learned and the action becomes gradually automated and only little attention is needed while performing an action. When this automatization happens, a motor skill has developed. Various brain structures, such as the motor, prefrontal and parietal cortices, the cerebellum and the basal ganglia are involved in sequencing skills. One brain structure that is involved in the development of a motor skill is the supplementary motor area (SMA). First reported by Penfield and Welch in 1949, this brain area still gets attention when it comes to studying the organization of voluntary movements. The present study is based on previous research by Verwey, Lammens and van Honk (2002) and Ruitenberg, Verwey, Schutter and Abrahamse (2014). They studied the development of a motor skill with the main focus on the SMA.

1.1 Anatomy

The SMA is assumed to be involved in movement preparation and execution. It is active during the performance of repetitive and sequential movements (Gerloff, Corwell, Chen, Hallett, & Cohen, 1997). The SMA can be divided into the pre-supplementary motor area (preSMA) and the supplementary motor area (SMAproper). Both brain areas are not only anatomically but also functionally closely connected (Figure 1). Nevertheless, different functionalities are attributed to the two brain areas.

The posterior-located SMAproper is directly connected to the primary motor cortex (M1), a brain area associated with movement, that has a direct connection to the spinal cord (Picard & Strick, 2001). The SMAproper is probably involved in simple movement and is probably responsible for loading and executing individual sequence elements. The preSMA is located in the rostro medial aspect of Brodmann's area 6 in the medial frontal cortex (Kennerly, Sakai, Rushwort, 2004), this area is closely connected with the prefrontal cortex and associated with cognitive inputs for motor behavior. The preSMA seems to be involved in cognitive and motor tasks, such as learning sequential movements and is assumed to be involved in complex movements (Picard & Strick, 2001). In particular, the preSMA is

responsible for retrieving motor chunks from memory and loading these motor chunks into the motor buffer. In short, the preSMA and SMAproper are both involved in the development of a motor skill but they have different roles regarding organization of movement. To measure the different functionalities a DSP task can be used.

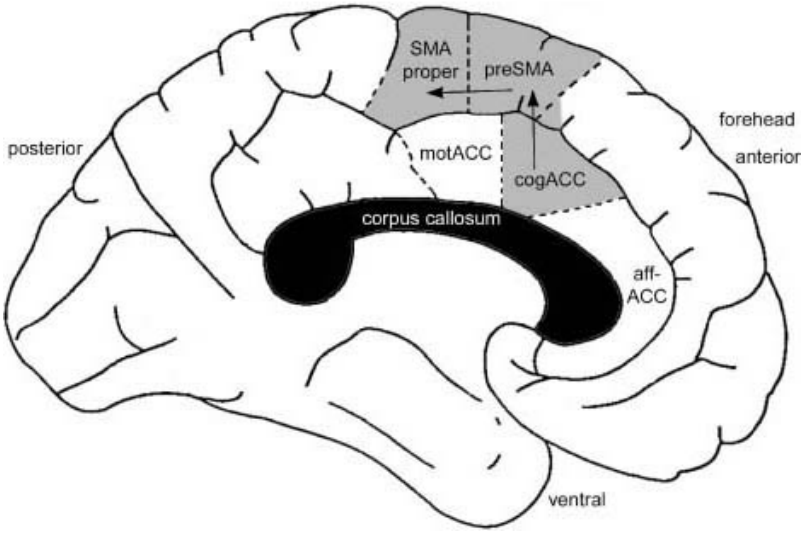

Figure 1. Visual presentation of the two brain areas SMAproper and preSMA (Alm, 2011).

1.2 DSP task

Developing a motor skill, such as learning how to shift gears or how to ride a bike, does not happen within a few hours. It takes time to develop a motor skill. Therefore, an experimental paradigm that enables the fast development of a motor skill, is needed. In this study, the simplest form of a motor skill, the execution of a short series of key presses, is chosen to investigate the neural basis of a motor skill (Ruitenberg et al., 2014).

A task that is well suited for studying this human capacity to acquire sequential a motor skill is the DSP task. This task provides insight in the development of voluntary sequential motor skills in humans (Ruitenberg et al., 2014).

In a classical DSP task, the participant is seated in front of a computer and places four to eight fingers on keys on a keyboard, and a similar number of placeholders is shown on the screen. These placeholders usually consist of small squares that light up and the participant presses the corresponding key on the keyboard as shown in figure 2. This results in sequences of three to seven stimuli. The task starts with a practice phase, wherein the participant repeats two sequences about 500 to 1,000 times (Ruitenberg et al., 2014).

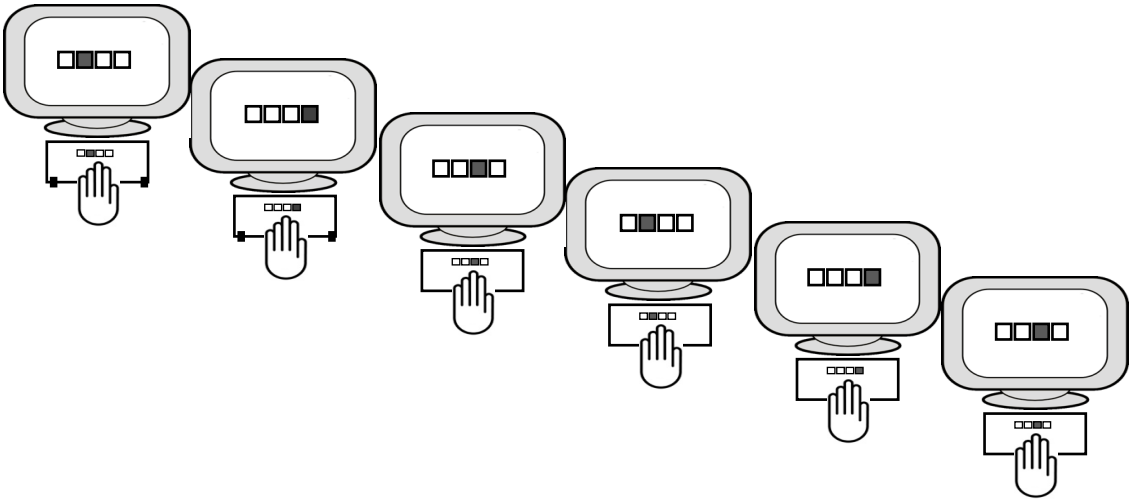

Figure 2. Representation of a DSP task comprising a sequence with 4 keys with the left hand

The cognitive processes involved in a sequentialmotor skill are addresses by the Dual processor model (DPM) (Abrahamse et al., 2013). The model states that two processors, a cognitive processor and a motor processor, are active while executing a sequential motor skill. Initially, the cognitive processor is responsible for translating a presented stimulus into a response and it prepares the motor processor to execute this response. Motor chunks are built, the cognitive processor selects these motor chunks and puts them into the so-called motor buffer. This triggers the motor processor, which then executes the movement. While performing sequences without any practice the cognitive processor is active. After some practice, the cognitive processor becomes less involved, and the motor processor can execute the motor chunks. After more practice, the execution of a movement becomes more and more automated, and the cognitive effort decreases. A sequential motor skill can be performed in three different modes of sequence execution: reaction mode, chunking mode and associative mode (Abrahamse et al., 2013). The reaction mode occurs when participants encounter the new sequence for the first time. The participant receives a stimulus and responds, receives another stimulus and responds, and so on. For each response a stimulus is needed in the reaction mode. During this stage of stimulus-based skill, the premotor cortex (PMC) is assumed to be highly active. In the associative mode, the participant still responds to stimuli but the response also primes the subsequent responses. After some practice, the participant starts to work in the chunking mode. The participant starts to divide the sequence into building blocks, so-called motor chunks. A sequence can be separated into several motor chunks depending on its length. The separation between motor chunks is called a

concatenation point. When performing the sequence, the first RT is relatively long, followed by the motor chunk, performed with short RTs. After the execution of the first motor chunk, the next motor chunk is loaded into the motor buffer, which is indicated by a long RT at this concatenation point. Then the next motor chunk is performed again with relatively short RTs (Abrahamse, Ruitenberg, de Kleine, & Verwey, 2013). In the chunking mode, the participant is able to perform the sequence solely based on the first stimulus of the motor chunk. From reaction mode to chunking mode, the motor skill shifts from stimulus-based to memory-based sequence performance. The activity of the PMC, which is active during the early stages of learning, decreases, whereas the activity of the SMA increases with practice. A method to measure the brain activity during sequence execution is transcranial magnetic stimulation.

1.3 TMS

Transcranial magnetic stimulation (TMS) is a non-invasive method for studying the human brain based on the principle of electromagnetic induction. Brain processes, such as motor function, language, vision, and pathophysiology of brain disorders can be investigated with this method (O'Shea & Walsh, 2007). A coil, connected to a pulse generator is placed on the scalp. The pulse generator delivers electric current and the coil produces a magnetic field via electromagnetic induction in specific brain areas (Miller, 2012). The effect of TMS is "to stimulate neuronal activity and change the excitation and organization of neuronal firing in the stimulated region" (O'Shea & Walsh, 2007, p.196). That means, for example, that the stimulation over the motor cortex can produce a muscle twitch and stimulation over the occipital cortex can produce visual phosphenes or scotomas (Hallett, 2000). The pulses of the stimulation can vary in intensity, frequency, and number and repetition of pulses. These factors influence whether the effects of TMS are excitatory or inhibitory (O'Shea & Walsh, 2007).

In the present study, TBS was used. TBS is a recently developed innovation of the classical TMS. Huang, Edwards, Rounis, Bhatia and Rothwell (2005) first introduced TBS. The classical TBS protocol by Huang et al. (2005) consists of three pulses that are applied at 50 Hz at 80% of the active motor threshold (AMT), repeated at intervals of 200ms (5 Hz) for 40 seconds. Continuous TBS (cTBS) is assumed to produce inhibitory after-effects, whereas intermittent (iTMS) and intermediate TBS (imTMS) are assumed to produce facilitatory after-effects (Huang et al. 2005). Furthermore, cTBS can be applied with 600 or 300 pulses per stimulation, with 600 pulses producing stronger and longer lasting-after-effects. So, in the present study, cTBS with 600 pulses was chosen. One of the biggest advantages of TBS over

TMS is the reduced stimulation time. TMS lasts about 20 to 45 minutes, whereas TBS just lasts about one to three minutes (Chung, Hoy, & Fitzgerald, 2015). Additionally, TBS is used with a high intensity and a high frequency, which results in longer-lasting after-effects (Huang et al., 2005). With cTBS with 600 pulses behavioral effects reduce to baseline activity after 60 minutes. The exact duration of the effects and whether the stimulation produces facilitatory or inhibitory effects hinges on the number of TBS pulses and other parameters such as intensity and repetition (Huang et al., 2005).

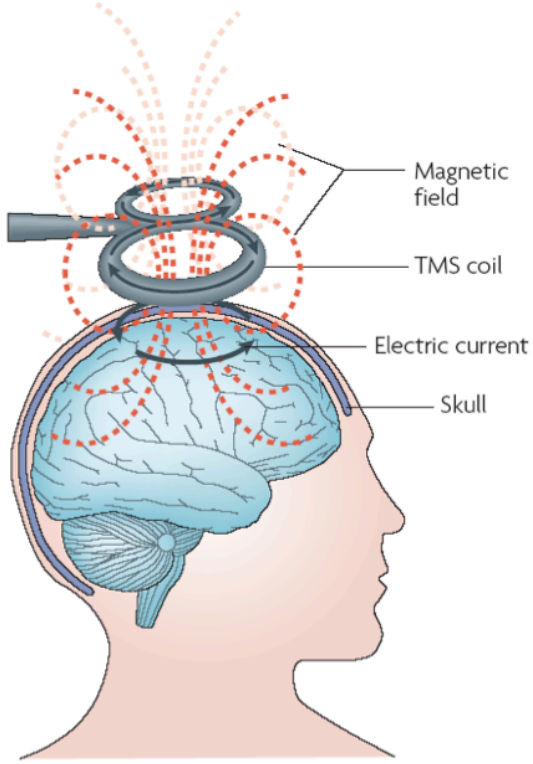

Figure 3. Visual illustration of TMS (Spronk, Arns & Fitzgerald, 2010).

1.4 Comparison of two previous studies

Verwey et al. (2002) and Ruitenberg et al. (2014) investigated the functionalities of the SMA during a DSP task. They have suggested distinct functional roles for the preSMA and the SMAproper in learning and producing motor sequences. However, the experimental design of the studies was not exactly the same.

The study by Verwey et al. (2002) assumed that the SMAproper is responsible for the performance of motor sequences; specifically, that the brain area is responsible for the execution of each element of a sequence. The experimental setup looked as follows: Twelve participants performed the DSP task and with the use of 20 min, 1Hz, rTMS, the SMAproper

was stimulated. When performing the DSP task after stimulation of the SMAproper the overall sequence completion time was lower than the sham condition. The mean RT for each response in the sequence was shorter with application of TMS.

The study by Ruitenberg et al. (2014) involved 48 participants. They also used 20 min, 1Hz, rTMS, but to stimulate the preSMA instead of the SMAproper. They found evidence that the preSMA is involved in the initiation of motor chunks and in dealing with the cognitive demands of sequence complexity, meaning that longer sequences are split into several motor chunks. Participants performed the DSP task with slowed RTs after TMS. In particular, the study revealed that motor chunk initiation was disrupted after the stimulation, not only at the start of each sequence, but also halfway through the sequence when a new chunk was supposed to be initiated at the concatenation point.

Although the procedures of the two studies were quite similar, some differences in the setup of the studies weakened the comparison (see Appendix A). The studies were carried out in different laboratories. This could be a problem due to unknown environmental factors that could have influenced the procedure and the outcomes of the studies. There were also some procedural differences. One was that the studies involved different numbers of blocks and trials in the practice and the test phase. That is, Verwey et al. (2002) used 420 trials divided over three blocks in the practice phase and four blocks with 40 trials in the test phase, whereas Ruitenberg et al. (2014) used 720 trials divided over eight blocks in the practice phase and four blocks of 60 trials in the test phase. Another procedural difference was that in the study by Verwey et al. (2002) participants performed the practice and the test phase in one day, and the next day the participants performed the practice and the test phase of the remaining condition, whereas in the study by Ruitenberg et al. (2014), the practice and test phase were split over two consecutive days. Furthermore, in the study by Verwey et al. (2002) participants practiced the sequences without the display of key-specific stimuli, whereas in the study by Ruitenberg et al. (2014) participants practiced with key-specific stimuli for both sequences. In addition, the test phase in the two studies was designed differently. Verwey et al. (2002) used only a familiar test condition and tested solely the chunking mode, meaning that participants practiced the same sequence they later had to perform in the test phase. Ruitenberg et al. (2014), meanwhile, used four different test conditions to test chunking mode and reaction mode. As in Verwey et al. (2002), there was a familiar test condition, where participants had to perform the sequence known from the practice phase based on a single stimulus (single stimulus condition) and in a second condition based on every key-specific stimulus (familiar guided condition). The third test condition consisted of familiar sequences

(familiar conditions) and the fourth test condition consisted of mixed unfamiliar sequences (mixed unfamiliar condition). It is possible, that the outcome of the comparisons of the respective brain areas is due to variations in the study protocols and not due to actual differences in the functionalities of the SMAproper and the preSMA. Therefore, the present study investigated the use of the SMAproper and the preSMA in the execution of the DSP task in a single laboratory, using identical DSP tasks protocols for each condition. In the present study, a stimulation with cTBS was chosen over rTMS because of the much shorter stimulation time and the high stimulus intensity, resulting in inhibitory after-effects (Huang et al., 2005).

1.5 Hypotheses

The aims of the present study are (1) to compare the different functionalities of the preSMA and the SMAproper in a single motor sequencing task; and (2) to evaluate the use of TBS to investigate the functionalities of the two brain areas after the stimulation.

It was assumed that stimulation with cTBS would produce the same inhibitory effects on the SMA areas as stimulation with TMS. It was expected that stimulation of the preSMA would slow down the RTs reflecting motor chunk initiation, but that the stimulation would have no effect on the RTs of the other responses. So, the initiation of the first motor chunk and the initiation of any additional motor chunks would be slowed down with the application of TBS on the preSMA. Furthermore, it was expected that participants would split up the 1x6 sequence into several motor chunks. This would replicate the findings of the studies by Verwey et al. (2002) and Ruitenberg et al. (2014).

It was expected that after stimulation of the SMAproper, each response to each stimulus of the sequence would be performed slower, resulting in overall longer RTs of both chunk initiation responses and other responses, as the study by Verwey et al. (2002) showed. It was inferred that there were no effects of stimulation on RTs in the sham condition.

2. Method

2.1. Participants

A total of 48 participants (20 male, 28 female) between 18 years and 44 years (mean age 26.3 ± 5.4) were included in the study. The participants were paid 40€ or were rewarded with study credits for participating in this study. Written informed consent was obtained from all participants.

According to a Power analysis with the G*Power 3.1 software (Faul, Erdfelder, Lang, & Buchner, 2007) a sample size of 16 participants per TBS condition would be adequate to achieve significant results. The Power analysis was based on the numbers (alpha level of 0.05, desired power of 0.8, effect size of 0.884) of the two previous studies by Verwey et al. (2002) and Ruitenberg et al. (2014).

All participants were classified as right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971), had good eyesight and indicated they were in a good mental and physical health condition. The exclusion criteria to participate in this study were in accordance with the general TMS safety guidelines. These guidelines include that the participants may not have a history or evidence of chronic or residual neurological disease, a pacemaker, deep brain stimulation, metal implants in the head or neck area or the chance of pregnancy (Rossi, Hallett, Rossini, Pascual-Leone, 2009). Furthermore, people with an alcohol, drug or tobacco addiction were excluded from the study.

The study was approved by the ethics committee of IfADo, the Leibniz Research Centre for Working Environment and Human Factors in Dortmund, Germany.

2.2. Apparatus

Stimulus presentation and response registration were controlled by the E-prime© 2.0 experimental software package that was programmed on a standard Windows 7 PC. The PC had an experimental mode, where all services that could have had an influence on the reaction time measurements were shut down. Stimuli were presented on a 21-inch LCD iiyama display. Responses were given on a standard qwertz-keyboard. TBS was delivered using a Mag & Moore PowerMAG Clinical pp TMS device stimulator with a standard, 70mm figure-of-eight double coil held by the researcher. A standard 70mm coil was used, because even

though smaller coils are found to produce more localized electric fields (Deng, Lisanby & Peterchev, 2013) the coil would overheat because of the high intensity and frequency of TBS.

2.3. The DSP task

Participants were instructed to place four fingers of their left hand on the keys *c, v, b* and *n* of a regular computer keyboard. Four horizontally aligned placeholders were displayed on the screen. When a placeholder on the screen lit up green, the participant responded as quickly as possible by pressing the corresponding key. Then the next placeholder lit up. In this way sequences of six stimuli were presented. In the practice phase the participant practiced two different sequences, one 2x3 sequence and one 1x6 sequence. A total of eight sequences were used to counterbalance the sequences across the participants. The following 1x6 sequences were carried out: BCVNVC, NVBCBV, CBNVBN, VNCBCN and the following 2x3 sequences NCBNCB, CVNCVN, VBCVBC and BNVBNV. Each sequence was practiced 720 times, divided across eight blocks. There was a 40-second break halfway through each practice block and a 4-minute break at the end of each practice block. In the test phase there were four blocks with 40 trials, 20 trials of a 2x3 sequence and 20 trials of a 1x6 sequence, counterbalanced across participants. The four test conditions were: familiar, single stimulus, unfamiliar and random. In the familiar test conditions, the participants had to perform the same two sequences, they already practiced in the practice phase. All key-specific stimuli of the sequence were displayed and the participant simply had to respond to them. In the single stimulus condition, the participants had to perform the same two sequences as in the previous test block but only the first stimulus of the sequence was displayed and the participant had to finish the sequence from memory. In the familiar test condition, the participants had to perform two, to them unknown, sequences and in the random test condition the participants had to perform sequences which occurred randomly. Due to the purely stimulus- based performance during execution of unknown sequences, the unfamiliar test block was assumed to cause PMC activity.

2.4. TBS

TBS was used to stimulate the preSMA and the SMAproper activity. The previous studies by Verwey et al. (2002) and Ruitenberg et al. (2014) have shown that the preSMA is located at 15% distance between the nasion and the inion anterior to Cz on the sagittal midline (Kennerly et al., 2004), and the SMAproper is located at 10% of this distance. A cross was

drawn on the participant's head to mark the position of either of the two areas. The mean distance between preSMA and SMAproper was 1.72cm. The activated TBS coil was placed above one of these areas; the mean distance between preSMA and Cz was 5.38cm and the mean distance between SMAproper and Cz was 3.68cm.

The participants were seated in a chair in a comfortable position with their eyes open. EMG electrodes were attached to the right hand over the abductor digiti minimi muscle (ADM). First, the motor hot spot was identified. This was done by holding the activated TMS coil over the hand area of M1 on the scalp and then searching for the coil position that resulted in the largest motor evoked potential (MEP) at a given intensity of the ADM. This point was then marked as the motor hotspot. Next, the ascending staircase method was applied (Schutter and van Honk, 2006), by increasing or decreasing the intensity of the stimulation systematically by 2%, starting at 45% intensity. This was done until the lowest stimulation frequency was defined, which resulted in clearly visible MEP amplitudes under moderate ADM contraction in half of all TMS stimuli. In this way, the intensity of the EMG was determined. Then, the active motor threshold (AMT) was determined by asking the participants to press their little finger at maximum force. The maximum force was displayed on a screen visible to the researcher and the participant. Next, 15% of the average maximum force was calculated, and the participants were asked to press their little finger with enough force to achieve t the calculated force value. The display of this force was again visible for the researcher and the participant. The participant was instructed to hold the finger on the 15% force. While the participant did so, the coil was again placed on the earlier marked hotspot on the scalp. The intensity, that gave a MEP signal that was bigger than the signal of the 15% of the maximum force was determined. 80% of this intensity was then calculated, and this intensity was used for the stimulation with TBS.

For the sham condition, the TBS coil was placed in a 90-degree angle on the preSMA or SMAproper. From earlier experience with TBS, it is known that some people can feel the stimulation. Therefore, the present study is a between-subject design. We chose this type of design for logistical reasons, to avoid cross-over learning effects, and to prevent participants from knowing in which condition they participated. Thus, the study design consisted of three different conditions, and participants in the sham condition did not know this was the case because they were not able to compare with other groups. The participants were randomly assigned to one of the three conditions (preSMA, SMAproper or sham condition).

2.5. Procedure

First the participants filled in the informed consent, the screening questionnaire, the Handedness Inventory and a demographic questionnaire to determine if they met the requirements to participate in the study. Then a medical check-up was performed to confirm that the participants met the physical and mental requirements. Participants were randomly allocated to the TBS groups.

After that, the hand area of the M1 and the AMT was determined. Next, the participant received instructions about the DSP task and began with the practice phase in a room with a computer and no other distractions. The participants practiced six of the eight practice blocks on the first day. They were allowed to take a break of four minutes between the practice blocks. On the consecutive day, the participant practiced the two remaining blocks of the practice phase. After practicing, the participants filled in the Awareness questionnaire, in which they were asked to recall the two sequences, to recognize them among 12 sequences, and eventually, if they noticed something during the practice phase and if they wanted to make a remark. After that, the participant was brought to the laboratory. There, depending on the condition the participant was assigned to, the position of the preSMA or the SMAproper was determined and marked on the participants' scalps. The participants received cTBS with 600 pulses of 5 Hz for 40 seconds (Huang et al., 2005). Thereafter, they walked from the laboratory where the stimulation took place to the laboratory with the setup for the DSP task, which was opposite to the first room. This took about 20 seconds, so nearly immediately after the stimulation, the test phase (T0) started. The practice phase and the test phase took place in the same room. The participant performed the four test blocks of the sequence task. There were four conditions, which were counterbalanced across the participants. The conditions were: familiar, familiar-single stimulus, unfamiliar and random. After 20 minutes, the participant again performed the four blocks (T20). The experiment lasted about three hours on the first day and one hour on the second day for each participant.

Table 1

Overview of the procedural steps in the present study.

Day 1	Completion of informed consent, screening and demographic questionnaire, Handedness Inventory
	Medical check-up
	Determination of hand area of M1 and AMT to define stimulation intensity
	Practice phase (Block 1-6)
Day 2	Practice phase (Block 7, 8)
	Completion of Awareness questionnaire
	Determination of preSMA/SMAproper
	Stimulation with TBS
	Test Phase (Block 9)
	Test Phase (Block 10)

3. Results

The TBS procedure was well tolerated by all participants and no surprising events occurred during the experiment. The data files resulting from the DSP task were merged with EMerge, cleaned with E-DataAid and analyzed with SPSS.

First, mean RTs within the 2x3 and 1x6 sequences were calculated for every participant in each block of the practice and the test phase. We defined RT as the time between stimulus presentation and depression of the appropriate response key in the familiar, unfamiliar and random test condition. In the single stimulus condition, we defined RTs as the time between key presses. Sequences with an erroneous response and the first two sequences of each block and the first two sequences after a break were excluded from the analyses, as were RTs deviating more than 2.5 times the standard deviation of the average RT of that sequence in each condition of a particular block across all participants. This last step removed 1.7% from the data.

3.1 Practice phase

A mixed ANOVA on RTs with Block (8), Sequence (2: 1x6 vs 2x3) and Key (6) as within-subject variables and TBS group (3: preSMA vs SMAproper vs sham) as between-subject variable showed an effect of Block, $F(7, 315) = 378.1$, $p < .001$, $\eta_p^2 = .80$, indicating that performance improved with practice. There was an effect of Sequence, $F(1,45) = 5.3$ $p < .026$, $\eta_p^2 = 0.11$. As shown in figure 4, the 2x3 sequence was performed faster than the 1x6

sequence, but this difference reduced with practice. There were no significant differences between the three TBS groups in performance, indicating no baseline differences between the three groups, $F(2,45) = 0.1$, $p = .960$. There was an effect of Key, $F(5,225)=172.9$ $p< .001$, $\eta_p^2= .79$, indicating that RTs of key presses differed. Further, there was an interaction of Block and Sequence, $F(7,315)=4.5$, $p< .001$, $\eta_p^2= .09$, showing that the difference between the sequences diminishes with practice. There was an interaction of Block and Key, $F(35, 1575)=44.3$ $p< .001$, $\eta_p^2= .50$ which shows that the difference between RTs of keys reduced over the eight practice blocks, and an interaction of Sequence and Key, $F(5, 225)=17.7$, $p< .001$, $\eta_p^2= .28$, indicating that sequence performance and RTs of the six keys differed between the two sequences.

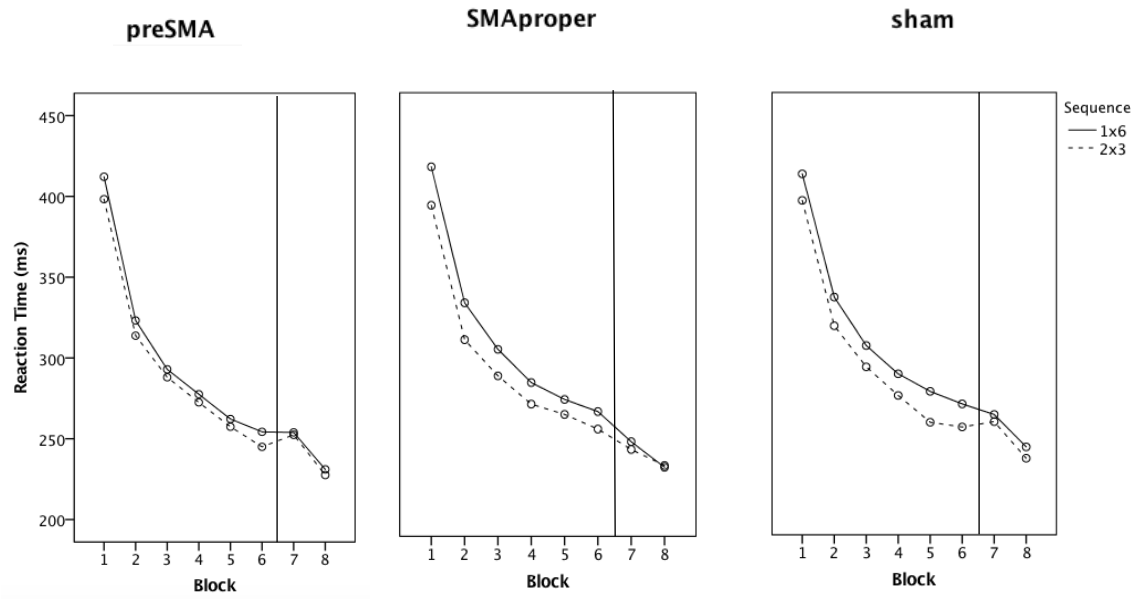

Figure 4. Learning curve of the eight practice blocks compared between the 1x6 and 2x3 sequence.

A mixed ANOVA on proportions of correctly performed sequences with Block (8) and Sequence (2) as within-subject variables and TBS group (3) as between-subjects variable revealed that there was a difference in performance among the eight blocks, $F(1,45)=13.9$, $p< .001$, $\eta_p^2 =.24$. This difference in accuracy differed from 94.1% accuracy in Block 1 to 95.7% accuracy in Block 8. There was no effect of sequence, $F(1,45)= 0.1$, $p =.764$, suggesting that accuracy did not differ significantly between the 1x6 and the 2x3 sequence. Also, there were no main or interaction effects of the TBS group (ps >.413), suggesting that there were no baseline differences.

3.2 Test phase

3.2.1 Overall analysis

Performing a mixed ANOVA on RTs with Time (2: T0 vs T20), Test Condition (4: familiar vs single stimulus vs unfamiliar vs random), Sequence (2: 1x6 vs 2x3) and Key (6) as within-subject variables and TBS group (3: preSMA vs SMAproper vs sham) as between-subject variable showed that performance in the four test conditions differed, $F(3,135)= 281.4$, $p< .000$, $\eta_p^2= .86$. RTs in the familiar condition were fastest with 243ms. RTs in the single stimulus condition were slower with 283ms, confirming the race between response selection and response triggering. RTs in the unfamiliar condition (407ms) and the random condition (452ms) were performed slowest, indicating a purely stimulus-based response in the random test condition. Further, the mixed ANOVA revealed that the 2x3 sequence (341ms) was performed faster than the 1x6 sequence (351ms) in three of the four test conditions. In the random condition, the 1x6 sequence was performed faster. The 2x3 sequence was performed faster in all three TBS groups, $F(1,45)= 55.9$, $p< .001$, $\eta_p^2= .55$. Additionally, there was an effect of Time, $F(1,45)= 45.0$, $p <.001$, $\eta_p^2= .50$, indicating that RTs were faster after 20 minutes delay than immediately after the stimulation (figure 5).

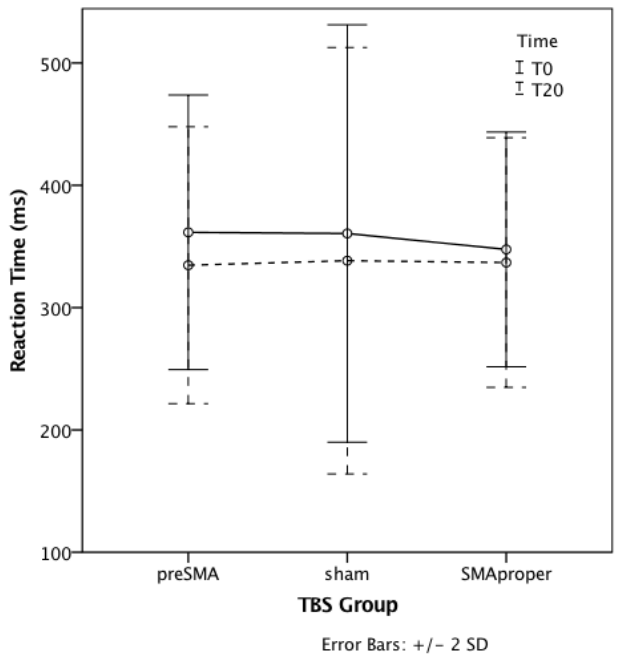

Figure 5. Comparison of performance at T0 and T20 between the three TBS groups across the four test conditions.

A Time and TBS group interaction suggested the effect of Time differed for the three TBS groups, $F(2,45)=3.2$, $p <.048$, $\eta_p^2= .13$. An interaction of Time and Test Condition, $F(3, 135)=29.7$, $p< .001$, $\eta_p^2= .39$ was seen. Further, there was an interaction of Time and Test Condition and TBS group, $F(6, 135)=2.6$, $p< .022$, $\eta_p^2= .10$, indicating that RTs differed in the four test conditions at T0 in the three TBS groups. Mean RT in the familiar and single stimulus condition seem shorter in the SMAproper group compared to the preSMA and sham group (figure 6). To further investigate if this observation shows any significant differences between the three TBS groups, a planned comparison at T0 was carried out. A one-way ANOVA was carried out to compare the performance of participants in the SMAproper group to the preSMA and sham group. There were no significant differences between the SMAproper group and the preSMA and sham group at T0, $F(2,45)= 498.1$, $p =.539$. There was an interaction of Time and Key, $F(5, 225)=4.9$, $p< .001$, $\eta_p^2= .09$, an interaction of Test Condition and Key, $F(15, 675)=55.1$, $p< .001$, $\eta_p^2= .55$ and an interaction of Sequence and Key, $F(5, 225)=29.7$ $p< .001$, $\eta_p^2= .39$, indicating that RTs of the six keys differed between Time, Test Condition and Sequence. There was no significant difference in RTs between the three TBS groups, $F(2,45)= 0.6$, $p= .540$.

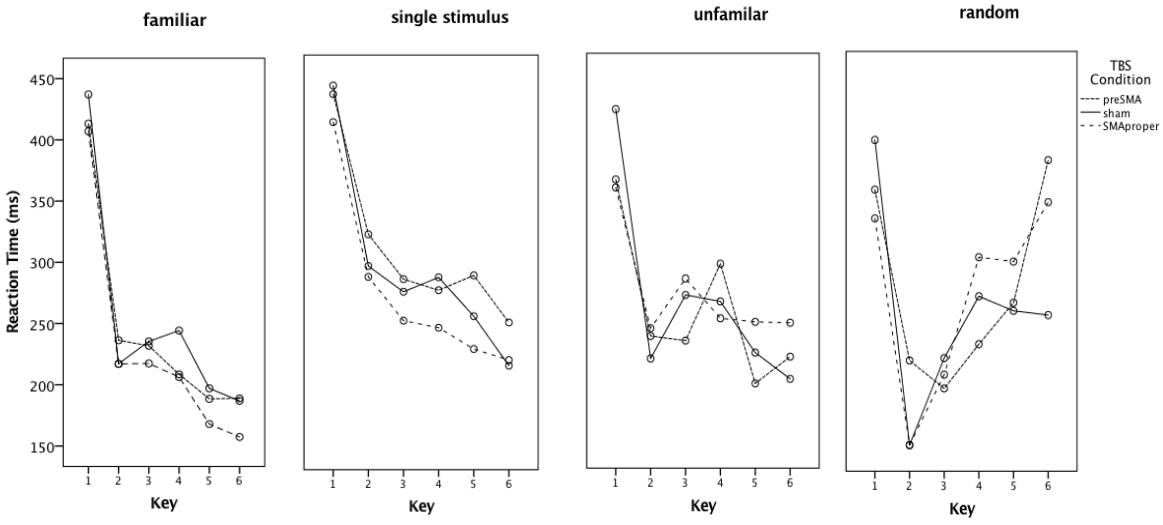

Figure 6. Comparison of performance in the four Test Conditions at T0 between the three TBS groups.

3.2.3 Motor chunk initiation

To examine if the initiation and execution of motor chunks differed amongst the three TBS groups, first, it was examined if participants segmented the two sequences into motor chunks. This was done with RTs of the last practice block (Block 8). It is assumed that the first key press reflects the initiation of the first motor chunk. The initiation of another motor chunk is reflected by a key within the sequence that is significantly slower than its preceding as well as its succeeding key. A one-tailed t-test per participant was carried out to examine chunk points (Ruitenberg et al., 2014) The second, third, fourth, fifth and sixth key were compared separately for the 1x6 and the 2x3 sequence. The one-tailed t-test revealed that 25 of the 48 participants segmented the 1x6 sequence into multiple motor chunks and 33 of the 48 participants segmented the 2x3 sequence into multiple motor chunks. RTs of key presses that were defined as the first key press of a motor chunk were averaged to calculate the mean initiation RT, and the remaining RTs were averaged to calculate the mean execution RT.

A mixed ANOVA with Phase (2; chunk initiation vs. execution of other keys), Time (2: T0 vs T20), Test condition (2; familiar vs single-stimulus) and Sequence (2; 1x6 vs 2x3) as within-subject variables and TBS group (3) as between-subjects variable was carried out. The ANOVA was based on the found chunk points in Block 8 and therefore only includes participants who did split up the sequences into motor chunks. A phase by test condition interaction indicated differences in motor chunk initiation in the two test conditions, $F(1,45)= 26.8$, p. <.001, $\eta_p^2= .37$. Motor chunk initiation was faster in the familiar test condition than in the single stimulus condition (373ms vs. 414ms). There was no significant difference in RTs of motor chunk initiation and execution between the three TBS groups, $F(2,45)=1.4$, $p=.263$ (figure 7).

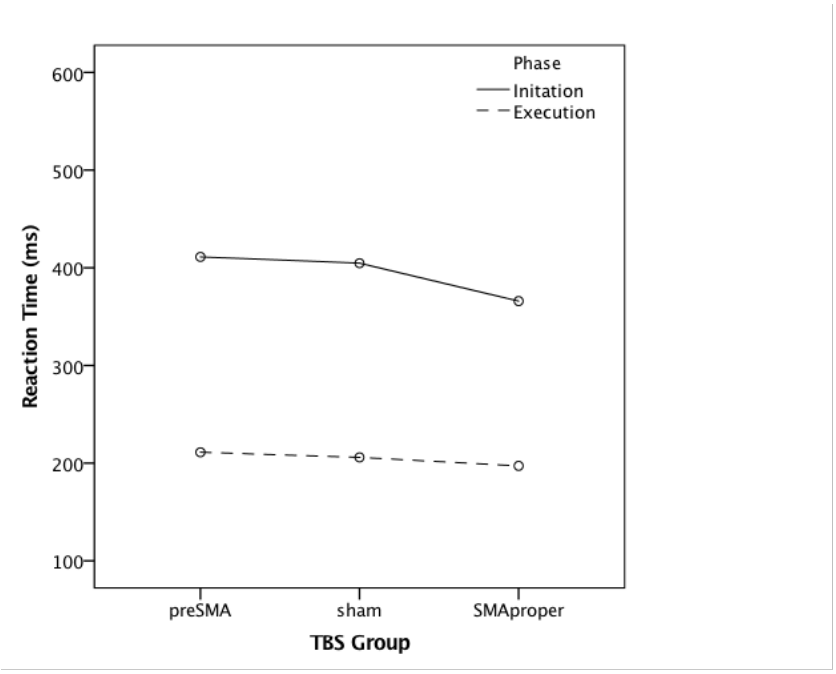

Figure 7. Chunk initiation and execution across the three TBS groups.

3.2.4 Accuracy

The proportion of correctly performed sequences was highest in the familiar condition with 94.2%, followed by 92.5% in the unfamiliar condition, 91.9% in the single stimulus condition, and 91.4% in the random condition. Accuracy in the 2x3 sequence was higher (93.2%) than in the 1x6 sequence (91.8%). A mixed ANOVA on the proportions of correctly performed sequences with Time (2), Test condition (4) and Sequence (2) as within-subject variables and TBS group (3) as between-subject variable revealed that the number of correct performed sequences differed among the four test conditions, $F(3, 45)= 8.3$, $p <.001$, $\eta_p^2 =.36$, with the highest accuracy in the familiar condition. Additionally, the analysis revealed an effect of Time and Sequence. Accuracy was higher in the test block performed with 20 minutes delay than in the test block immediately after the stimulation, $F(1,45)= 6.2$, $p <.017$, $\eta_p^2= .12$, and in the 2x3 sequence compared to the 1x6 sequence, $F(1,45)= 5.0$, $p <.03$, $\eta_p^2= .01$. Additionally there was an interaction effect of Time and Test condition, $F(1,45)= 8.3$, $p<.006$, $\eta_p^2=.12$. There were no differences in accuracy between the three TBS Groups, $F(2,45)=3.2$, $p =.728$

3.3 Explicit sequence knowledge

The evaluation of the awareness questionnaire showed no differences in recall or recognition of the 1x6 or 2x3 sequence. That means that the differences in explicit sequence knowledge in table 2 cannot be attributed to group differences.

Table 2

Explicit sequence knowledge

The numbers and the corresponding percentages of participants per group (16 participants per group) who correctly wrote down their 1x6 and 2x3 sequences immediately after the practice phase (recall columns) and recognized their sequences from a set of 12 alternatives (recognition columns).

	Recall		Recognition	
	1x6	2x3	1x6	2x3
preSMA	7 (43.8%)	9 (56.3%)	12 (75%)	13 (81.3%)
SMAproper	9 (56.3%)	13 (81.3%)	14 (87.5%)	15 (93.5%)
Sham	7 (43.8%)	9 (56.3%)	15 (93.8%)	12 (75%)

4. Discussion

The present study had two objectives. First, the present research sought to support the idea of different functionalities of preSMA and SMAproper; second it aimed to evaluate the use of TBS in this experimental design. The experimental protocols of the previous studies by Verwey et al. (2002) and Ruitenberg et al. (2014) were combined to one protocol with the objective of investigating the roles of the preSMA and the SMAproper in one experimental design. We applied cTBS at 5 Hz for 40 seconds either to the preSMA or the SMAproper and compared participants' performance on a DSP task with each other and with the performance of participants who received a sham stimulation. It was expected that stimulation of the preSMA would result in a slowing of chunk initiation and that stimulation of the SMAproper would result in a slowdown of all RTs. This would have been in line with the findings of the studies by Verwey et al. (2002) and Ruitenberg et al. (2014). However, this study could not replicate the findings of the two previous studies, and the hypotheses are not supported by the findings of this study. Stimulation of the preSMA did not result in a significant lower RT for chunk initiation than chunk execution.

Furthermore, there was no difference of overall RTs between the three groups. Participants carried out the 2x3 sequence faster than the 1x6 sequence and the fastest performance was measured in the familiar test condition and in the single stimulus test condition. The slowest performance was measured in the random test condition followed by the unfamiliar test condition. RTs were higher 20 minutes after the stimulation than immediately after the stimulation. But, there was no difference in performance between the preSMA, SMAproper and sham group after TBS.

Nevertheless, immediately after the stimulation there was a performance difference between the three TBS Groups in the four Test Conditions. It seems that participants who received stimulation of the SMAproper showed shorter RTs in the familiar and single stimulus condition compared to participants in the preSMA and sham group. This small difference could indicate a facilitatory effect of TBS on the SMAproper. But with detailed analysis no significant difference was found. Still, this small RT differences could indicate a minor facilitatory effect of the stimulation on the SMAproper. This is inconsistent with the findings of Verwey et al. (2002), who found an inhibitory effect of TMS on the SMAproper.

These results are inconsistent with the expectations of this study, but there are several findings in this study worth mentioning. First, one of the differences with the two previous studies is the change of the kind of stimulation. The previous studies stimulated either the preSMA or SMAproper with 20 minutes rTMS. TMS is able to inhibit motor execution (Verwey et al., 2002, O'Shea & Walsh, 2007, Hallett, 2000, Ruitenberg et al., 2014). In the present study an innovative development of the classical TMS was used. TBS is shorter and applied with a higher intensity than TMS. It was expected to show the same results as studies with TMS did. That this was not the case could be due to the differences between TMS and TBS and the required experimental setup. Intensive literature research gave some insight into possible explanation for the absence of TBS effect.

Zielmann et al. (2008) and Wischnewski and Schutter (2015) have indicated that effects of stimulation with TBS could be influenced by muscle contraction before, during, and after the stimulation. The experimental setting in this study let participants walk from the room where they practiced the DSP task to a laboratory, where the stimulation took place, back to the room where they performed the test phase of the DSP task. The distance of the room and the laboratory was small, about five meters, and it took no more than 20 seconds to walk from one room to another, but it could have influenced the effects of the stimulation through the muscle contractions that come with walking before and after the stimulation. As

Ziemann et al. (2008) and Wischnewski and Schutter (2015) have stated, more research on the effect of muscle contraction regarding the effects of TBS is needed to make more reliable assumptions.

Additionally, Ziemann et al. (2008) have stated that there is too little information about the effect of changing the parameters of the stimulation, such as the intensity or the number of pulses per burst, and it is not certain that the protocol of this study, which copied the original TBS protocol by Huang et al. (2005), was the best choice for this kind of research.

Regarding spatial resolution, O'Shea and Walsh (2007) have stated that with TMS it is possible to stimulate different brain areas, and a distance of 0.5 to 1cm is sufficient to distinguish between different areas. The mean distance between the preSMA and SMAproper, in this study, was 1.7cm, so it would be expected that this distance was large enough not to stimulate both areas at the same time. Deng et al. (2013) found that a figure-eight type coil, that is used in the present study, was able to stimulate a determined brain area as precise as possible, compared to other types of coils. But there is little known about the sufficient distance when using TBS. It was expected that the focality with TBS would be as good as with TMS. But it could be that a stimulation with TBS reaches further than a stimulation with TMS, and that the two areas are anatomically too closely connected, so that while stimulating one, the other area was affected by the pulses as well. Also, no research about the efficacy of a figure-eight type coil while using TBS could be encountered. This would explain why there were no differences between the preSMA and the SMAproper group, but it would not explain the missing differences in the sham group. Future research could use anatomical or functional MRI to confirm the localization of the stimulation site to improve the localization of the brain areas.

Differences in the setup of the study could also led to the absence of aftereffects. The present study and the study by Ruitenberg et al. (2014) involved 720 practice trials per sequence, whereas the study by Verwey et al. (2002) only used 210 practice trials per sequence. Both studies found significant effects when stimulating either the preSMA or the SMAproper. It could be that TBS was not strong enough to disrupt the learning effect of 720 trials per sequence and with fewer practice trials, there would have been a significant effect.

There was another difference regarding the experimental design. In Verwey et al. (2002) rTMS was applied by a fixed-coil position, and in Ruitenberg et al. (2014) the coil was movement-corrected. A robot arm was used that held the coil on the earlier determined area on the scalp. When the participants made a small movement, the robot arm also moved to

hold the coil in the right position. However, in the present study, the coil was handheld by the researcher without movement correction or fixation other than the hand. This could have led to an unstable fixation with little movement that could have caused insufficient stimulation.

A third difference regarding the experimental design that could have had an influence is the difference in practicing the sequences in Verwey et al. (2002) and Ruitenberg et al. (2014). In Verwey et al. (2002) participants had to learn two sequences by heart at first and then practice them by reacting to the display of only the first stimulus. When stimulating the SMAproper the functionality became inhibited and resulted in lower overall RTs. In contrast, in Ruitenberg et al. (2014) participants practiced the sequences guided by the display of all key-specific stimuli. When stimulating the preSMA, only chunk initiation becomes inhibited. When presented with key-specific stimuli other brain areas could take over the role of the preSMA or SMAproper and could react to a stimulus, which could explain the slowing of chunk initiation and not of execution of other keys, leading execution to be unaffected. When presented with a single stimulus, this takeover is not possible, and inhibition of SMAproper activity slows all RTs. Perhaps, this design difference is responsible for the assumed difference in functionality of the preSMA and SMAproper.

Nevertheless, there are several studies that have used TBS and shown significant effect on behavioral outcomes. For instance, a study by Huang et al. (2002) used continuous TBS with 300 pulses and found significant changes in RTs when stimulating the motor cortex for 40 seconds with 600 pulses of continuous TBS. But the present study was the first study that did research on the preSMA and the SMAproper in combination with the use of TBS; no prior research with these specific attributes could be encountered. A scientific review about the efficacy of TBS in humans (Wischnewski & Schutter, 2015) has shown that the effects of TBS differ between cortical regions. This could mean, that the present study is one of the first studies that reports the failure of TBS when it comes to the stimulation of the supplementary motor areas. To support these findings, future research is needed to exclude other factors that could have influenced this outcome.

For future research a follow-up study with a few improvements is suggested. Using another measure to confirm the localization of the preSMA and SMAproper would give more certainty about the right localization and targeting of the stimulation. Furthermore, using both, TMS and TBS, to stimulate the preSMA and SMAproper could give more insight into the differences of the two TMS methods. Moreover, it would be interesting to investigate the difference regarding the practice of the sequences with or without key-specific stimuli. In a

future study, a group that practices the sequences without key-specific stimuli and another group that practices with them could investigate the functionalities of the preSMA and SMAproper. When comparing the performance of both groups after stimulation, the question remains as to whether the assumed differences in functionality of supplementary motor areas are caused by procedural differences or whether they really exist.

One finding of the present study is support for the assumption of the DPM model by Abrahamse et al. (2013). This study has provided evidence that sequences are executed in the three different modes: the reaction mode, the associate mode, and the chunking mode, and that the initiation of a motor chunk can be differentiated from the execution of other keys, indicated by higher RTs. Additionally, there is support for the notion of a cognitive and a motor processor racing to produce the next response, indicated by the fastest RTs in the familiar test condition.

4.1 Conclusions

The findings of the studies by Verwey et al. (2002) and Ruitenberg et al. (2014) could not be replicated in the present follow-up study. This can probably be attributed to the fact that TBS stimulation had no significant effect on the functions of the preSMA and SMAproper in the present motor-sequencing task. This study provides reasons for the assumption that TBS is unable to inhibit the functions of the supplementary motor areas enough to show a significant effect on performance. For future research a follow-up research with some adjustments regarding the experimental setup of the study is suggested. It would be interesting to use TMS and TBS to compare their maybe different effects on the brain areas. Additionally, a localization method for the brain areas should be used, the participants should get less practice time and muscle contraction before, during and after the stimulation should be avoided. In this way the experimental setup of the present study can be improved and use for future research to get more insight into the functionalities of preSMA and SMAproper and the use of TBS.

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Appendix

A: Differences and Similarities between the three studies

	Verwey, W. B., Lammens, R., & van Honk, J. (2002)	Ruitenberg, M. F. L., Verwey, W. B., Schutter, D. J. L. G., & Abrahamse, E. L. (2014)	ten Kate, L. P., Ruitenberg, M. F. L., Kuo, M., Jamil, A., Kato Rand, M., Nitsche, M & Verwey, W. B. (2017)
Sequencing Task	Single-stimulus in practice and test phase (pure chunking mode)	Key specific stimuli in practice and test phase (chunking mode plus reaction mode)	Key specific stimuli in practice and test phase (chunking mode plus reaction mode)
	No RSI (max. Execution rate)	50 ms RSI in practice and test phase (reduced execution rate)	No RSI (max. Execution rate)
	Only single stimulus in practice and test phase, no display of key-specific stimuli	Display of key-specific stimuli during practice phase	Display of key-specific stimuli during practice phase
	210 practice trials/sequence	720 practice trials/sequence	720 practice trials/sequence
Test Phase	Test condition: only familiar single-stimulus	Test condition: familiar guided/ familiar single-stimulus/ familiar mixed (with deviants)/ unfamiliar mixed	Test condition: familiar guided/ familiar single-stimulus/ unfamiliar/random
	Test block included 40 trials	Test block included 60 trials	Test block included 4 times 40 trials
Stimulation	SMA vs sham: within subjects	preSMA vs. rPMC vs. Sham: between subjects	SMA vs. preSMA vs sham: between subjects
	SMA at 10% distance between inion and nasion, anterior to Cz	preSMA at 15% distance between inion and nasion, anterior to Cz	SMA at 10% distance between inion and nasion, anterior to Cz preSMA at 15% of this distance

	rTMS: fixed coil position	rTMS: Movement corrected	TBS: handheld coil position
	Stimulation: 90% of motor threshold	Stimulation: 90% of motor threshold	Stimulation: 80% of active motor threshold
	20 min TMS	20 min TMS	40 s TBS
Timing	Test partly on day 2/limited consolidation	Test entirely on day 2/full consolidation	Test entirely on day 2/ full consolidation
	Test condition: post-TMS: 0, 20, 25	Only 20 min post-TMS	0 and 20 min post-TBS
	Compare post-TMS with pre-TMS test block	Compare various post TMS test blocks (20 min TMS)	Compare different conditions, compare post TBS test blocks
Similarities	Same counterbalanced sequences 2 sequences differing in complexity (2x3 and 1x6) left hand same four keys: CVBN		

B: Questionnaires used in the study

Demographic Questionnaire:

Teilnehmerfragebogen

Teilnehmernummer:
Alter:
Geschlecht:
Beruf:

a) Videospiele

Führen Sie folgende Aktivität regelmäßig aus?	Seltener als einmal pro Woche	1-7 Stunden pro Woche	1-7 Stunden pro Tag

Wie lange führen Sie die Aktivität schon aus?	Seit weniger als einem Jahr	Seit 1-5 Jahren	Seit mehr als 6 Jahren

Wie lange ist es her, dass Sie die Aktivität ausgeführt haben?	Weniger als ein Monat	Bis zu 3 Jahre	Mehr als 3 Jahre

b) Klavier spielen

Führen Sie folgende Aktivität regelmäßig aus?	Weniger als einmal pro Woche	1-7 Stunden pro Woche	1-7 Stunden pro Tag

Wie lange führen Sie die Aktivität schon aus?	Seit weniger als einem Jahr	Seit 1-5 Jahren	Seit mehr als 6 Jahren

Wie lange ist es her, dass Sie die Aktivität ausgeführt haben?	Weniger als ein Monat	Bis zu 3 Jahre	Mehr als 3 Jahre

Screening Questionnaire

Teilnehmernummer:

Screening Fragebogen für Teilnehmer an der TBS Studie

1. Haben Sie Epilepsie oder hatten Sie jemals einen Anfall?	Ja/Nein
2. Sind Sie jemals ohnmächtig geworden? Wenn ja, beschreiben Sie bitte die Umstände.	Ja/Nein
3. Hatten Sie jemals eine ernste Verletzung am Kopf? (mit anschließender Bewusstlosigkeit)	Ja/Nein
4. Haben Sie Problem mit den Ohren oder Ohrensausen?	Ja/Nein
5. Sind Sie schwanger oder besteht die Möglichkeit einer Schwangerschaft?	Ja/Nein
6. Haben Sie Metall im Kopf? (z.B. Splitter, Clips etc.)	Ja/Nein
7. Haben Sie ein Cochlea Implantat?	Ja/Nein
8. Haben Sie einen implantierten neuro- Simulator? (z.B. DBS, epidermal/subduraal, VNS)	Ja/Nein
9. Haben Sie einen Schrittmacher oder andere Drähte im Herzen, oder Metall im Körper?	Ja/Nein
10. Haben Sie ein Infusion System für Medikamente?	Ja/Nein
11. Nehmen Sie Medikamente? (bitte aufschreiben)	Ja/Nein
12. Hatten sie jemals eine Operation am Rückenmark?	Ja/Nein
13. Haben Sie Dränagen oder Ventrikel?	Ja/Nein
14. Hatten Sie schon einmal eine TMS Untersuchung?	Ja/Nein
15. Hatten Sie jemals einen MRT Scan?	Ja/Nein

Handedness Questionnaire

Teilnehmernummer:
Edinburgh Händigkeitsinventar
Bitte sagen Sie uns, welche Hand Sie bei den unten genannten Tätigkeiten bevorzugen, indem Sie ein + in das entsprechende Kästchen setzen. Wenn Sie bei einer Tätigkeit ausschließlich die eine Hand nehmen und niemals die andere, kennzeichnen Sie dies bitte durch zwei + + . Wenn Sie sich nicht entscheiden können, welche Hand Sie bevorzugen, setzen Sie bitte ein + in beide Kästchen.
Bitte versuchen Sie, alle Fragen zu beantworten.

Welche Hand nehmen Sie ...		**linke** Hand	**rechte** Hand
1	... zum Schreiben?		
2	... zum Malen?		
3	... zum Werfen?		
4	... zum Schneiden mit der Schere?		
5	... zum Zähneputzen?		
6	... wenn Sie ein Messer halten (ohne Gabel)?		
7	... wenn Sie einen Löffel halten?		
8	... nach oben, wenn Sie einen Besen halten?		
9	... um das Streichholz zu halten, wenn Sie es anzünden?		
10	... um den Deckel von einer Schachtel zu nehmen?		

Awareness Questionnaire:

Teilnehmernummer:

Es folgen drei Fragebögen. Sie dürfen:
- eine Seite des Fragebogens erst lesen, wenn Sie fertig mit der vorherigen Seite sind.
- eine Seite nicht mehr verändern, wenn Sie die folgende Seite gelesen haben.

Fragebogen 1

In diesem Experiment haben Sie durch das Drücken einer Taste auf das leuchtende Viereck auf dem Monitor reagiert. Es gab zwei feste Abfolgen in denen die Vierecke während des ganzen Experiments aufleuchteten, somit gab es auch zwei Tastenabfolgen.

Können Sie für *beide* Abfolgen angeben welche aufeinanderfolgenden Tasten Sie gedrückt haben (mit Verwendung der Tasten CVBN)?

C	V	B	N

Bild der vier Tasten auf der Tastatur

Eine Abfolge war: _____

Wie sicher sind Sie sich, auf einer Skala von 1 (absolut unsicher) bis 10 (absolut sicher) dass diese Abfolge richtig ist?

_____

Die andere Abfolge war: _____

Wie sicher sind Sie sich, auf einer Skala von 1 (absolut unsicher) bis 10 (absolut sicher) dass diese Abfolge richtig ist?

Drehen Sie diese Seite um, wenn Sie fertig sind – Sie dürften nicht mehr umblättern!

Fragebogen2

In der nachfolgenden Tabelle stehen 12 Abfolgen mit 6 Tasten. Ihre Abfolge ist auch dabei. Versuchen Sie anzugeben, welche zwei Abfolgen Sie gedrückt haben. Schreiben Sie anschließend auf, wie sicher Sie sich bei Ihrer Entscheidung sind (1: absolut unsicher, 10: absolut sicher)

C	V	B	N

Bild der vier Tasten auf der Tastatur

Reihenfolge								‘X’, für Ihre Abfolge	*1: absolut unsicher 10: absolut sicher*
1	V	C	B	C	N	V			
2	B	C	V	N	V	C			
3	N	V	B	C	B	V			
4	C	N	V	B	C	N			
5	V	N	C	B	C	N			
6	N	B	C	V	N	B			
7	B	N	V	B	N	V			
8	C	B	N	V	N	B			
9	B	V	C	V	B	N			
10	N	C	B	N	C	B			
11	C	V	N	C	V	N			
12	V	B	C	V	B	C			

Fragebogen 3

1) Wie haben Sie Ihre Abfolge in den zwei vorangehenden Fragebögen erkannt? (eine Antwort einkreisen)
Da ich mich an die Buchstabenfolge erinnerte
Ich erinnerte mich an die Position der Tasten/ der Vierecke auf dem Monitor
Da ich mit meinen Fingern die Abfolge in Gedanken nachspielte
Anders, nämlich:

2) Haben Sie schon einmal an einem anderen Experiment teilgenommen bei dem es um Tastenkombinationen ging?
Waren es die gleichen Abfolgen?

3) Ist Ihnen etwas aufgefallen oder haben Sie Anmerkungen zu dem Experiment?

C: SPSS Syntax Practice phase

```
DATASET ACTIVATE DataSet2.
GLM B1_1x6_Key1 B1_1x6_Key2 B1_1x6_Key3 B1_1x6_Key4 B1_1x6_Key5
B1_1x6_Key6 B1_2x3_Key1 B1_2x3_Key2
   B1_2x3_Key3 B1_2x3_Key4 B1_2x3_Key5 B1_2x3_Key6 B2_1x6_Key1 B2_1x6_Key2
B2_1x6_Key3 B2_1x6_Key4
   B2_1x6_Key5 B2_1x6_Key6 B2_2x3_Key1 B2_2x3_Key2 B2_2x3_Key3 B2_2x3_Key4
B2_2x3_Key5 B2_2x3_Key6
   B3_1x6_Key1 B3_1x6_Key2 B3_1x6_Key3 B3_1x6_Key4 B3_1x6_Key5 B3_1x6_Key6
B3_2x3_Key1 B3_2x3_Key2
   B3_2x3_Key3 B3_2x3_Key4 B3_2x3_Key5 B3_2x3_Key6 B4_1x6_Key1 B4_1x6_Key2
B4_1x6_Key3 B4_1x6_Key4
   B4_1x6_Key5 B4_1x6_Key6 B4_2x3_Key1 B4_2x3_Key2 B4_2x3_Key3 B4_2x3_Key4
B4_2x3_Key5 B4_2x3_Key6
   B5_1x6_Key1 B5_1x6_Key2 B5_1x6_Key3 B5_1x6_Key4 B5_1x6_Key5 B5_1x6_Key6
B5_2x3_Key1 B5_2x3_Key2
   B5_2x3_Key3 B5_2x3_Key4 B5_2x3_Key5 B5_2x3_Key6 B6_1x6_Key1 B6_1x6_Key2
B6_1x6_Key3 B6_1x6_Key4
   B6_1x6_Key5 B6_1x6_Key6 B6_2x3_Key1 B6_2x3_Key2 B6_2x3_Key3 B6_2x3_Key4
B6_2x3_Key5 B6_2x3_Key6
   B7_1x6_Key1 B7_1x6_Key2 B7_1x6_Key3 B7_1x6_Key4 B7_1x6_Key5 B7_1x6_Key6
B7_2x3_Key1 B7_2x3_Key2
   B7_2x3_Key3 B7_2x3_Key4 B7_2x3_Key5 B7_2x3_Key6 B8_1x6_Key1 B8_1x6_Key2
B8_1x6_Key3 B8_1x6_Key4
   B8_1x6_Key5 B8_1x6_Key6 B8_2x3_Key1 B8_2x3_Key2 B8_2x3_Key3 B8_2x3_Key4
B8_2x3_Key5 B8_2x3_Key6 BY
   TBSCondition1
  /WSFACTOR=Block 8 Polynomial Sequence 2 Polynomial Key 6 Polynomial
  /METHOD=SSTYPE(3)
  /PLOT=PROFILE(Block*Sequence Block*TBSCondition1)
  /EMMEANS=TABLES(TBSCondition1) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Block) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Sequence) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Key) COMPARE ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Block Sequence Key Block*Sequence Block*Key Sequence*Key
Block*Sequence*Key
  /DESIGN=TBSCondition1.
```

D: SPSS Syntax Test phase

```
* Encoding: UTF-8.

DATASET ACTIVATE DataSet1.
GLM T0_fam_1x6_Key1 T0_fam_1x6_Key2 T0_fam_1x6_Key3 T0_fam_1x6_Key4
T0_fam_1x6_Key5 T0_fam_1x6_Key6
   T0_fam_2x3_Key1 T0_fam_2x3_Key2 T0_fam_2x3_Key3 T0_fam_2x3_Key4
T0_fam_2x3_Key5 T0_fam_2x3_Key6
   T0_singstim_1x6_Key1 T0_singstim_1x6_Key2 T0_singstim_1x6_Key3
T0_singstim_1x6_Key4
   T0_singstim_1x6_Key5 T0_singstim_1x6_Key6 T0_singstim_2x3_Key1
T0_singstim_2x3_Key2
   T0_singstim_2x3_Key3 T0_singstim_2x3_Key4 T0_singstim_2x3_Key5
T0_singstim_2x3_Key6
   T0_unfam_1x6_Key1 T0_unfam_1x6_Key2 T0_unfam_1x6_Key3 T0_unfam_1x6_Key4
T0_unfam_1x6_Key5
   T0_unfam_1x6_Key6 T0_unfam_2x3_Key1 T0_unfam_2x3_Key2 T0_unfam_2x3_Key3
T0_unfam_2x3_Key4
   T0_unfam_2x3_Key5 T0_unfam_2x3_Key6 T0_rand_1x6_Key1 T0_rand_1x6_Key2
T0_rand_1x6_Key3
   T0_rand_1x6_Key4 T0_rand_1x6_Key5 T0_rand_1x6_Key6 T0_rand_2x3_Key1
T0_rand_2x3_Key2
   T0_rand_2x3_Key3 T0_rand_2x3_Key4 T0_rand_2x3_Key5 T0_rand_2x3_Key6
T20_fam_1x6_Key1
   T20_fam_1x6_Key2 T20_fam_1x6_Key3 T20_fam_1x6_Key4 T20_fam_1x6_Key5
T20_fam_1x6_Key6
   T20_fam_2x3_Key1 T20_fam_2x3_Key2 T20_fam_2x3_Key3 T20_fam_2x3_Key4
T20_fam_2x3_Key5
   T20_fam_2x3_Key6 T20_singstim_1x6_Key1 T20_singstim_1x6_Key2
T20_singstim_1x6_Key3
   T20_singstim_1x6_Key4 T20_singstim_1x6_Key5 T20_singstim_1x6_Key6
T20_singstim_2x3_Key1
   T20_singstim_2x3_Key2 T20_singstim_2x3_Key3 T20_singstim_2x3_Key4
T20_singstim_2x3_Key5
   T20_singstim_2x3_Key6 T20_unfam_1x6_Key1 T20_unfam_1x6_Key2
T20_unfam_1x6_Key3 T20_unfam_1x6_Key4
   T20_unfam_1x6_Key5 T20_unfam_1x6_Key6 T20_unfam_2x3_Key1
T20_unfam_2x3_Key2 T20_unfam_2x3_Key3
   T20_unfam_2x3_Key4 T20_unfam_2x3_Key5 T20_unfam_2x3_Key6
T20_rand_1x6_Key1 T20_rand_1x6_Key2
   T20_rand_1x6_Key3 T20_rand_1x6_Key4 T20_rand_1x6_Key5 T20_rand_1x6_Key6
T20_rand_2x3_Key1
   T20_rand_2x3_Key2 T20_rand_2x3_Key3 T20_rand_2x3_Key4 T20_rand_2x3_Key5
T20_rand_2x3_Key6 BY
   TBSCondition
  /WSFACTOR=Time 2 Polynomial TestCondition 4 Polynomial Sequence 2 Polynomial Key
6 Polynomial
  /METHOD=SSTYPE(3)
```

```
  /PLOT=PROFILE(TestCondition*Sequence TBSCondition*Time)
  /EMMEANS=TABLES(TBSCondition) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Time) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(TestCondition) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Sequence) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Key) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Time TestCondition Sequence Key Time*TestCondition Time*Sequence
TestCondition*Sequence
    Time*TestCondition*Sequence Time*Key TestCondition*Key Time*TestCondition*Key
Sequence*Key
    Time*Sequence*Key TestCondition*Sequence*Key Time*TestCondition*Sequence*Key
  /DESIGN=TBSCondition.


GLM T0_fam_1x6_initiation T0_fam_2x3_initiation T0_singstim_1x6_initiation
    T0_singstim_2x3_initiation T20_fam_1x6_initiation T20_fam_2x3_initiation
    T20_singstim_1x6_initiation T20_singstim_2x3_initiation T0_fam_1x6_execution
T0_fam_2x3_execution
    T0_singstim_1x6_execution T0_singstim_2x3_execution T20_fam_1x6_execution
T20_fam_2x3_execution
    T20_singstim_1x6_execution T20_singstim_2x3_execution BY TBSCondition
  /WSFACTOR=Phase 2 Polynomial Time 2 Polynomial TestCondition 2 Polynomial
Sequence 2 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Phase) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Time) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(TestCondition) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Sequence) COMPARE ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Phase Time TestCondition Sequence Phase*Time Phase*TestCondition
Time*TestCondition
    Phase*Time*TestCondition Phase*Sequence Time*Sequence Phase*Time*Sequence
TestCondition*Sequence
    Phase*TestCondition*Sequence Time*TestCondition*Sequence
Phase*Time*TestCondition*Sequence
  /DESIGN=TBSCondition.

ONEWAY T0_fam_1x6_Key1 T0_fam_1x6_Key2 T0_fam_1x6_Key3 T0_fam_1x6_Key4
T0_fam_1x6_Key5
    T0_fam_1x6_Key6 T0_fam_2x3_Key1 T0_fam_2x3_Key2 T0_fam_2x3_Key3
T0_fam_2x3_Key4 T0_fam_2x3_Key5
    T0_fam_2x3_Key6 T0_singstim_1x6_Key1 T0_singstim_1x6_Key2
T0_singstim_1x6_Key3 T0_singstim_1x6_Key4
    T0_singstim_1x6_Key5 T0_singstim_1x6_Key6 T0_singstim_2x3_Key1
T0_singstim_2x3_Key2
    T0_singstim_2x3_Key3 T0_singstim_2x3_Key4 T0_singstim_2x3_Key5
T0_singstim_2x3_Key6
```

```
   T0_unfam_1x6_Key1 T0_unfam_1x6_Key2 T0_unfam_1x6_Key3 T0_unfam_1x6_Key4
T0_unfam_1x6_Key5
   T0_unfam_1x6_Key6 T0_unfam_2x3_Key1 T0_unfam_2x3_Key2 T0_unfam_2x3_Key3
T0_unfam_2x3_Key4
   T0_unfam_2x3_Key5 T0_unfam_2x3_Key6 T0_rand_1x6_Key1 T0_rand_1x6_Key2
T0_rand_1x6_Key3
   T0_rand_1x6_Key4 T0_rand_1x6_Key5 T0_rand_1x6_Key6 T0_rand_2x3_Key1
T0_rand_2x3_Key2
   T0_rand_2x3_Key3 T0_rand_2x3_Key4 T0_rand_2x3_Key5 T0_rand_2x3_Key6
T0_fam_1x6_mean T0_fam_2x3_mean
   T0_singstim_1x6_mean T0_singstim_2x3_mean T0_unfam_1x6_mean
T0_unfam_2x3_mean T0_rand_1x6_mean
   T0_rand_2x3_mean BY TBSCondition1
  /STATISTICS DESCRIPTIVES EFFECTS
  /MISSING ANALYSIS
  /POSTHOC=BONFERRONI ALPHA(0.05).
```