Bachelor Thesis

The role of the Supplementary Motor Area during internally and externally triggered movement sequences: A TMS Study.

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Date: 22-08-2012

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Abstract

The present study was designed to address the interaction of cognitive and neurological correlates that are involved in the control of sequential motor skill. Responding to individual stimuli in unfamiliar sequences is thought to occur in the reaction mode, whereas familiar sequences are executed in the chunking mode. The supplementary motor area (SMA) is particularly engaged in the preparation and execution of highly practiced sequences that do not necessitate visual cues. We expected the chunking mode to be influenced by the SMA, due to the involvement of both in internally triggered action. First, participants performed eight practice blocks consisting of two discrete sequences in the DSP task. Second, the experimental group received 20 minutes of 1 Hz rTMS over the SMA; the control group did not receive any stimulation. Finally, participants performed a test block consisting of familiar, unfamiliar and single-stimulus sequences that only provided the first cue. We found that participants performed sequences of the unfamiliar test phase in the reaction mode and sequences in the familiar and single-stimulus test phase in the chunking mode. Our hypothesis that the experimental group showed a slowed performance compared to the control group during familiar and single-stimulus test phases could not be confirmed. It was found that during the single-stimulus test phase, groups differed in RTs on the first key. The SMA seems to play a key role in the preparation of sequences performed in the chunking mode, especially sequences that only provide the first stimulus. Only the control group benefitted from explicit sequencing knowledge.

(Nederlandstalige) samenvatting

Dit onderzoek richt zich op het samenspel tussen cognitieve processen en hersengebieden die een rol spelen tijdens het uitvoeren van sequentiële bewegingen. Eerdere onderzoeken lieten zien dat onbekende sequenties in de reactie modus en bekende sequenties in de chunking modus worden uitgevoerd. De supplementaire motorische cortex (SMA) is vooral betrokken bij het voorbereiden en controleren van vaak geoefende sequenties die geen visuele stimuli meer nodig hebben. Wij verwachtten dat de chunking modus aan de SMA gekoppeld kon worden aangezien allebei betrokken zijn bij intern aangestuurde bewegingen. Ten eerste voerden de proefpersonen gedurende acht oefenblokken twee discrete sequenties in de DSP taak uit. Ten tweede ontving de experimentele groep 20 minuten van 1 Hz rTMS op de SMA; de controle groep kreeg geen stimulatie. Tot slot voerden de proefpersonen één test blok uit die uit bekende, onbekende, en single-stimuli fasen bestond waarbij alleen de eerste cue getoond werd. Uit dit onderzoek bleek dat proefpersonen de bekende sequenties in de chunking modus en de onbekende sequenties in de reactie modus uitgevoerd hebben. Onze hypothese dat de experimentele groep langzamer op stimuli in de bekende en single-stimulus testfasen reageert dan de controle groep kon niet bevestigd worden. Er werd gevonden dat de experimentele groep een langere preparatiefase nodig had tijdens de single-stimulus fase. De SMA lijkt dus bij te dragen aan het voorbereiden van sequenties die in de chunking modus uitgevoerd worden, vooral sequenties die alleen de eerste stimulus tonen. Alleen de controle groep kon gebruik maken van expliciete kennis over de sequenties om hun reactietijden te verbeteren.

1. Introduction

Mastering motor skills is an ability that is essential to our everyday life because it reduces our need for constant attention and helps us deal with upcoming information. The main objective of this thesis is to identify the interaction of cognitive and neurological correlates that engage in the control of sequential motor skill. Specifically, we aim to link the sequence processing mode *chunking* to the supplementary motor area (SMA) by inhibiting the SMA and observing the consequences during different processing modes of sequential skills.

1.1 Motor Skills

From infancy onward we practice voluntary motor skills that help us interact with the world. We hereby move from mastering gross movements like posture to gradually learning to control fine movements like tying our shoe laces or writing a letter (Stallings, 1973). As we become more familiar with certain actions they tend to become more automatic and require less mental effort (Graybiel, 2008). For instance, reversing a car for the first time demands substantially more effort compared to the ease of an experienced driver. As most of the tasks we perform in everyday life consist of several smaller movements that are combined into larger actions, it is particularly useful to be able to master skills that consist of motor sequences. For example, playing an instrument requires the coordination of multiple steps that convergently lead to the desired result. Sequence learning can be studied in order to gain information about skill acquisition and control. During a sequence task participants repeat pressing certain character strings that, unbeknown to the participants, consist of a fixed sequence. After several practice sessions, participants recognize the sequential pattern and can usually execute it even without external instructions that tell them which keys to press (Verwey, 2002). The Discrete Sequence Production (DSP) task is one example of a sequence task that is commonly used to teach participants sequences and to draw conclusions about human skill acquisition and control (c.f. p.11).

It is important to consider different processing modes that are involved in the control of sequential motor skills and to take into account how they are represented by the brain. In the next section, three cognitive modes are discussed that are responsible for the production of sequential action during various levels of skill development.

1.2 Cognitive Modes of Sequencing Skill

According to Verwey (2003) sequential motor skill acquisition is marked by two distinct processes: When people first start learning sequences they need to pay attention to every visual stimulus of the task at hand. Without those external stimuli they would be unable to execute the sequence correctly because they would miss the cues that guide their actions. The performance in the beginning of the learning process is rather slow because participants respond to each stimulus individually. This processing mode is referred to as *reaction mode* (Verwey, 2003). After extended practice, participants are relatively fast at performing their sequences due to the development of motor chunks. According to Wymbs, Bassett, Mucha, Porter, & Grafton (2012), motor chunking aids movement production by combining individual motor elements into integrated units. Halford, Wilson, & Phillips (1998) stated that individual movements are then executed as a single program which demands less cognitive engagement. The brain thus takes a long string of motor information and converts it into a larger representation, a motor chunk. When participants are able to execute the entire sequence merely based on the first stimulus, they are thought to perform sequences in the *chunking mode*. Subsequent stimuli can be drawn from memory and are therefore no longer required (Verwey, 1999). Rosenbaum, Kenny, and Derr (1983) confirmed the occurrence of chunking by finding that there was a substantially higher amount of errors between chunks as compared to mistakes made within a chunk. This result contributes to the notion that elements were seen as a coherent unit (chunk) instead of separate elements.

The development from reaction to chunking mode can also be illustrated by the following example: Typing on a keyboard demands a great deal of attention when people do it for the first time. We are then highly dependent on the letters that are imprinted on the keys and guide our action. At first, it takes a lot of effort to search for each letter until we found it. This search can be linked to the reaction mode because it is dependent on sensory cues and requires a large amount of attention. With more practice, we gradually get familiar with the keyboard which speeds up the typing process because we know where to find the keys. After a while, we do not require external guidance anymore, we can type without looking at the keys or the screen anymore. This processing mode corresponds to the chunking mode due to its automatism and speed.

Recently there was found evidence for a possible third processing mode involved in the development of motor skills, the so-called associative mode, which is thought to fall in between

reaction and chunking mode (Verwey & Abrahamse, 2012). Motor action is not fully automatic yet, however, participants start associating the following stimulus and benefit from the priming by previous stimuli (Verwey, Abrahamse, & de Kleine, 2010).

Verwey (2001) described the processing shift from unfamiliar to familiar sequences in terms of a dual processor model (DPM) that illustrates the interaction of two functionally distinct systems, the cognitive processor and the motor processor. Studies showed that the cognitive processor is mostly involved in the preparation of familiar sequences whereas the motor processor is responsible for executing the series (Verwey et al., 2010). It was found that the cognitive processor loads a so-called motor buffer with sequence information about motor chunks that can be read by the motor processor, a process referred to as buffer loading (Henry & Roger, 1960).

During the reaction mode (i.e. when participants execute unfamiliar sequences) the cognitive processor prepares movements by loading individual sequential elements into the motor buffer, whereas the motor processor reads these elements from the buffer and executes them. After practice, the motor buffer is repeatedly filled with the same sequential elements, resulting in the development of motor chunks (Verwey, 1996). After motor chunks are formed, the motor processor is able to execute the sequences on its own. When participants execute familiar sequences and motor chunks were formed, the motor processor executes the chunk as a unit upon appearance of the first stimulus which acts as a cue. It is thought that the cognitive processor is the key element during the initial acquisition of motor skills during which the reaction mode is dominant. Accordingly, the motor processor seems to correspond to the chunking mode due to its automatic and internal nature. However, the cognitive processor can still assist during the chunking mode and hence remains accessible during the whole sequencing process (Verwey et al., 2010). The difference between unfamiliar and familiar sequences according to the DPM is the demand on the cognitive processor which is higher during the reaction mode and lower during the chunking mode (Verwey, 2001). Verwey and Abrahamse (2012) further proposed that the associative mode, which is characterized by a relatively modest improvement and a constant dependency on external stimuli, may develop before motor chunks but is not a necessary requirement for the development of such. In line with the DSM, they assumed that associations may enhance the ability of the cognitive processor to select successive responses.

1.3 Supplementary Motor Area

The transformation from initial motor learning to a chunking routine can also be observed in neural activity. A structure crucially involved in sequence learning is the supplementary motor area (SMA), which is the main focus of the present study. The SMA was formerly thought to consist of two areas, rostral and caudal SMA, which are also referred to as pre-SMA and SMA proper (from now on called SMA), respectively (Tanji, 1996). However, due to their functional differences, SMA and pre-SMA are nowadays known as two separate structures (Nachev, Kennard, & Husain, 2008). Due to its location in the medial portion of Brodmann's area 6, the SMA is able to project directly to both the primary motor cortex (M1) and the spinal cord which directly relates the SMA to motor output (Tanji, 1994). The SMA is part of the cortico-basal ganglia loop which is thought to be important for chunking (Graybiel, 2008) and selecting correct movements (Kandel, Schwartz, & Jessell, 2000).

The prominence of the SMA during sequential tasks was investigated by researchers who found that SMA activity increased when motor tasks involved sequential movements (Lee & Quessy, 2003). Studies conducted by Kornhuber and Deecke (1965) revealed that SMA activity increased right before the actual movement is executed, suggesting its involvement in motor preparation (see also: Deiber, Ibanez, Sadato, & Hallett, 1996; Roland, Larsen, Lassen, & Skinhej, 1980). Additionally, studies of Kennerley, Sakai, and Rushworth (2002), Tanji (1994), and Verwey, Lammens, & Van Honk (2002) point to the SMA as being involved in the temporal organization of sequences. Moreover, Amassian, Cracco, Cracco, & Maccabee (1990) showed that a temporary inhibition of the SMA via TMS can disturb sequential movements and bimanual coordination. Together, these studies provide evidence that the SMA is involved in the preparation and execution of sequential movement.

Hikosaka et al. (1999) suggested a striking resemblance between cognitive processing modes and neurological correlates by demonstrating that the SMA is especially active during the performance of already learned routines and less active during the execution of new sequences. This makes it reasonable to assume that the SMA plays a central role in the execution of familiar sequences which are retrieved from motor memory (Tanji, 1994). In the following section, the differences between internal and external retrieval are further explained.

1.4 Internally vs. Externally Triggered Movements

Retrieving sequences is a process that can be supported by visual cues, but can also be performed from memory. Internally triggered movements are movements that are executed independently of external assistance, and are hence drawn from memory. Familiar sequences that are performed in the chunking mode do not necessitate external guidance because their pattern was internalized. In contrast, externally triggered movements are movements that are supported by external cues that tell the participant what to do. External cues are especially helpful in the reaction mode during the beginning of sequential learning because they guide actions and facilitate the transition to the chunking mode.

While sequential action that is guided by visual cues is presumably performed by the premotor cortex (PMC) (Mushiake, Inase, & Tanji, 1991), there is accumulating evidence that the SMA is engaged in the execution of sequences under internal control. Halsband, Matsuzaka, & Tanji (1994) found that lesions in the SMA led to the inability to reproduce sequences from memory. Passingham (1993) further concluded that the SMA was particularly responsive to internally generated movement that was executed without external cues. Moreover, activity in the SMA increased when participants imagined or executed a memorized sequence which again points to the SMA as being associated with self-initiated internal movement (Roland et al., 1980). Animal studies confirmed that monkeys whose SMA was damaged experienced a performance decline when it came to sequences that lacked external cues. Monkeys subsequently were unsure about how to behave and could not complete the previously memorized sequence unless they were given sensory signals (Passingham, 1993). The GABA agonist[1] Muscimol that was injected bilaterally into the SMA had the same deteriorated effects during memory retention tasks that however could be solved by providing a visual signal. In sum, the SMA appears to play a key role in the retrieval and performance of sequential movements based on memory.

However, a simple dichotomy hypothesis about in-/externally triggered movements and their analogous neural substrates is discarded by many authors who found contradictory results, such as the finding that both SMA and PMC are equally active during sequence retrieval regardless of the presence of sensory signals (Okano & Tanji, 1987; Romo & Schultz, 1987; Kurata & Wise, 1988; Thaler, Rolls, & Passingham, 1988; as cited in Tanji et al., 1994). In more

[1] GABA agonist refers to a drug which increases the action at the GABA receptor which is the main inhibitory neurotransmitter in the central nervous system

recent years however, Kennerley et al. (2002) as well as Mushiake et al. (1991) demonstrated that most SMA neurons were exclusively active during a sequential task that needed to be retrieved from memory rather than being active in both internal and external conditions. So, it seems that the SMA is mainly involved in the production of internally guided sequential movements. Given its origin in the hippocampus and limbic system this is a rather logical assumption. The hippocampus is primarily involved in memory formation and storage which could indicate that the SMA is well suited to remember acquired motor action and can thus proceed with their execution despite a lack of external cues (Kandel et al., 2000).

1.5 The Present Study

This study examined whether the chunking mode can be linked to the SMA due to the internally triggered action that is associated with both the cognitive and neural correlates (i.e. chunking mode and SMA, respectively). Although contradictory studies casted doubt on the affiliation of the SMA with internally triggered movement, it appears to be a topic worth investigating in order to clarify the exact role of the SMA and its association with the chunking mode.

To investigate the assumed connection during sequential action, TMS was applied to influence the activity of targeted areas by interfering with local cortical function (e.g., see Lefaucheur, 2006). TMS is a non-invasive technique that causes depolarization or hyperpolarization in the neurons of the brain. Depolarization is a change in the neuron's membrane potential that makes it more positive, it thus excites the affected neuron. In contrast, hyperpolarization makes the membrane potential more negative, resulting in the inhibition of upcoming action potentials. TMS causes de- or hyperpolarization by inducing electric currents which change the magnetic field of the affected neuron. In the present study, TMS was used to hyperpolarize (i.e. inhibit) neuronal function of the SMA. If participants whose SMA was inhibited were unable to perform a certain task, it is thought that this task would usually (non-inhibited) involve the SMA. One can thus draw conclusions about the function of a given structure by inhibiting it and observing the consequences. Besides single pulses that are induced only once there is also the opportunity to make use of repetitive TMS (rTMS). The advantage of rTMS is the temporal summation of several pulses which can affect targeted areas substantially longer and presumably more effectively (Gerloff, Corwell, Chen, Hallett, & Cohen, 1997).

Accordingly, rTMS was used in this study in order to depress cortical excitability of the SMA. It was presumed that rTMS at a low frequency (20 min/1Hz) inhibits the SMA and thus leads to impaired execution of sequences that are performed in the chunking mode, but not the reaction mode.

The task implemented to prompt sequence execution in this study was the Discrete Sequence Production (DSP) task. In the present DSP task, participants repeatedly practiced two discrete sequences, each consisting of six elements. During the task, four horizontally aligned square placeholders were displayed on the screen. When a sequence began, one of the squares was filled with a green color, indicating that participants must respond by pressing the spatially corresponding key on their keyboard. As soon as a correct response was given, the next square lit up and again the corresponding key must be pressed. The end of the sequence was indicated by a temporary break between the last stimulus of the ongoing sequence and the first stimulus of the following sequence. Whereas in the beginning of the experiment the execution of the DSP task was externally triggered and dependent on key-specific stimuli, we expected that the task became more internally controlled after several practice blocks. The improvement after extensive practice can be attributed to the development of motor chunks (Verwey, 1996).

The DSP task is well suited for sequential learning because it allows for a fast development of sequencing skill in a controlled setting. Several studies using the DSP task demonstrated that after extended practice sessions, participants performed their sequences in the chunking mode (Verwey et al., 2010; Verwey, Abrahamse, Ruitenberg, Jiménez, & De Kleine, 2011). It was found that participants were able to proceed with the pattern even when only the first stimulus was displayed. They were thus able to retrieve the remaining responses from memory. Furthermore, a secondary task that was introduced during the aforementioned study did not distract the participants from executing the practiced movement (Verwey et al., 2010). It is thus highly likely that practicing sequences in the DSP task encourages participants to perform in the chunking mode. Additionally, the task was eligible for the purpose of this study because the sequences have a clear beginning and end, which makes it possible to study preparatory effects and gives the participant a clear idea of when to commence and stop executing sequences (Rhodes, Bullock, Verwey, Averbeck, & Page, 2004). Moreover, the DSP task was appropriate because its sequence length is limited. According to Miller (1956), human's working memory can hold 7 ±2 items at once. Since the DSP task that was used consists of 6 elements, it enables

participants to identify the number of sequences and recognize the chunking pattern that can be utilized.

Participants in the present study completed a two hour practice session on the first day of the experiment, during which they performed two sequences in the DSP task. They continued to practice the keying sequences on the second day for two blocks in order to brush up their sequencing skills from the day before. Shortly after finishing the DSP task, participants underwent 20 minutes of 1 Hz rTMS which was applied over the SMA, or they were assigned to a sham condition in which no actual stimulation occurred.[2] After a break of 20 minutes participants completed the DSP test phase, during which they performed both the previously practiced sequences as well as new and otherwise modified sequences.

Verwey (1996, 2001) suggested that task difficulty contributes to differences in the emergence of motor chunks. According to the DPM, motor chunks facilitate buffer loading which results in a faster execution of the motor processor. In order to investigate possible task difficulty effects, participants in the present study practiced a 6-key-sequence (1x6) as well as a paired 3-key-sequence, consisting of two successive instances of one 3-key segment (2x3). We expected lower task difficulty for the 2x3 sequence because it consisted of a repetition of two identical key segments. The 1x6 sequence did not consist of repetitions but of six loose keys that did not include repetitions. The reason for lower task difficulty experienced for the 2x3 sequence was either the double exposure (1x6 sequences are less practiced) and/or the decreased difficulty (De Kleine & Verwey, 2009). Differences in task difficulty were thought to be reflected in higher RTs during more difficult sequences.

It was hypothesized that after the practice phase participants would perform their sequences in the chunking mode. After the induction of rTMS over the SMA one could assume that this region would be temporarily inhibited and consequently, its involvement during sequence performance in the DSP test phase would be reduced. This would supposedly lead to a performance decline during the familiar test phase, because participants would be unable to incorporate the SMA which is responsible for initiating the chunking mode during the familiar sequence. The response time of participants in the experimental condition during the familiar test phase would hence be significantly longer compared to the control group where the function of

[2] The present study is part of a bigger study which additionally includes an experimental group with rTMS over the PMC. However, this is not discussed in the current work and therefore will not be mentioned again.

the SMA remained unaffected. In addition to the familiar test phase, there was also a single-stimulus test phase, consisting of only the first cue and thus asking participants to retrieve the remaining chunk elements from memory. We assumed that participants in the experimental group would make more errors during the single-stimulus phase because their ability to use the SMA would be reduced. Since they could not fall back on any visual stimuli (as in the familiar test mode) they were expected to have trouble completing the sequence internally which would lead to a greater error rate as compared to the control group. However, it is also possible that explicit sequence knowledge can compensate for the inhibition of the SMA.

Moreover, we expected participants in the experimental group to require a longer preparation time during familiar and single-stimulus test phases. Since the SMA is highly involved in sequence preparation, we expected that the experimental group whose SMA was affected would have to make more effort to initiate sequences performed in the chunking mode. Preparation for sequence execution is thought to be reflected in the mean RT on the first key, which is the phase classified as sequence initiation (Verwey, 2010). Furthermore, it was expected that the execution of unfamiliar sequences would not lead to a difference in response time between groups. Since sequences during the unfamiliar test phase made use of the initial cognitive mode (i.e. reaction mode) that is not thought to be predominantly controlled by the SMA, the rTMS induction should not impair sequencing performance.

2. Methods

2.1 Participants

A total of 19 participants (4 male, 15 female) took part in the present study. They were aged between 18 and 28 years (M=20.9) and were students at the University of Twente. All participants were classified as being right-handed according to Annett's Handedness Inventory (1970) and reported to have good eye sight (corrective glasses or contact lenses were permitted). Exclusion criteria in accordance with TMS guidelines were: history of neurological or hearing disorders, severe medical conditions, pacemaker or other metals located near the head, pregnancy, alcohol/drug consumption 48 hours/2 months prior to the experiment, and smoking history (c.f. Rossi et al., 2009). Participants gave their written informed consent and could receive credits they

needed to obtain as part of a course requirement. The experiment was approved by the Medical Ethical Committee (METC) of the Medical Spectrum Twente (MST).

2.2 Apparatus

Stimulus presentation and response registration were controlled by the E-prime© 2.0 experimental software package that was programmed onto a standard Pentium© IV Windows XP© PC. Windows services that could have delayed the reaction time measurement accuracy were shut down. Stimuli were presented on a 17-in Philips 107 T5 display. Responses were given on a standard QWERTY-keyboard.

Transcranial Magnetic Stimulation was applied using a high power Magstim Rapid 2 Stimulator©, connected to a figure-of-eight air-cooled coil that was held by an industrial robot (Viper s850 Six-Axis robot© from Adept Technology Inc.). The robot was controlled by the Advanced Neuro Technology (ANT) software program SmartMove©.

2.3 Task and Procedure

Before signing up for the study, participants were informed about the procedure via email and had the opportunity to ask questions and consider their participation. After confirming their attendance, they were asked to appear on two consecutive days with a 24 hour break in between both days. During the first day they practiced two keying sequences in the DSP task. The second day contained a two block practice session, the TMS treatment, and the test phase.

2.3.1 DSP Practice Phase

Upon entering the test room located at the Faculty of Behavioral Sciences at the University of Twente, participants were seated in front of the computer and informed about the upcoming procedure. In order to once again verify eligible participants, they were asked to fill out a screening questionnaire that filtered participants possessing possible exclusion criteria that would make them unable to continue the experiment. Moreover, Annett's Handedness Inventory was completed in order to ensure that only right handed participants took part. After they had the opportunity to ask questions, participants signed an informed consent form of which they could keep a copy for personal records. Participants were instructed to place their little, ring, middle, and index fingers of their left hand on the C, V, B, and N keys of the keyboard (see Figure 1).

The task consisted of responding to each stimulus that was presented as a green light appearing in one of the four boxes at a time by pressing the spatially corresponding key on the keyboard.

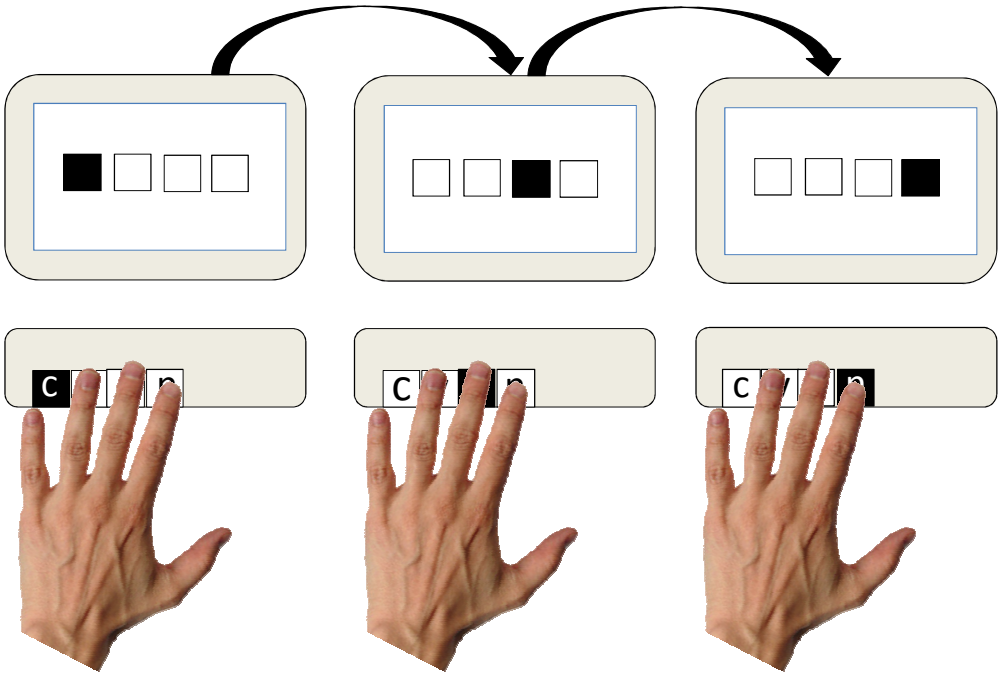

Figure 1. Demonstration of presented stimuli on the display that spatially corresponded to keys on a standard QWERTY keyboard.

Each of a total of six practice blocks included 180 trials (90 of each sequence), making for 540 repetitions of each sequence. When the participant pressed the correct key, the following stimulus lit up and again required the participant to respond with the corresponding key. After 6 stimuli were executed correctly, sequence completion was indicated by a break of 1000 ms before the first stimulus of the following sequence appeared. Pressing a false key led to an error message for 2000 ms which was used to motivate participants to avoid mistakes. The ongoing sequence was aborted. For all participants there was a 40 s resting period halfway through each practice block. Each of the eight practice blocks was also followed by a rest period of three minutes, during which participants received feedback about their mean response time and error rate.

Participants practiced one sequence that consisted of a paired-3-segment sequence (2x3) as well as one sequence consisting of 6 key presses that did not include repetitions. Keys were rotated across sequential positions in order to avoid that one finger contributed considerably more than another. The two sequences were selected out of 12 possible key-press combinations and counterbalanced across participants.

After completion of the sixth practice block on day one of the experiment, participants were asked to fill out an awareness questionnaire that tested their explicit knowledge of the practiced sequences. Firstly, they were instructed to recall their practiced sequences by writing down the consecutive keys of each sequence. Secondly, participants were asked to recognize "their" sequences from a list with 12 possible key-press combinations. They were also asked to indicate how certain they were about their choice on a scale from one to ten, ranging from "very uncertain" to "very certain", respectively. Finally, participants answered questions about their strategy regarding their memory recall. The duration of the practice phase was about two hours.

2.3.2 Repetitive TMS

The second day of the experiment took place at the Experimental Centre for Technical Medicine (ECTM) at the University of Twente. Two additional practice blocks were introduced as a short warm-up activity for two reasons: First, to make sure that participants memorized the sequences from the day before and second, to direct their minds towards the upcoming task.

After completing the DSP task, participants were asked to remove any jewellery or piercings that might interfere with the magnetic stimulation. Participants were then seated in a dental chair that allowed them to sit in a comfortable position while relaxing their legs. They were informed about the upcoming procedure and were given the opportunity to ask questions. Next, the appropriate intensity of stimulation had to be determined for each participant.

In order to do so, we first determined the motor hotspot, which was defined as the location on the primary motor cortex that evoked 100% responsitivity in the participant's hand (i.e. each pulse elicited a visible response). Criteria for responsitivity were a distinct and clear movement of the participants' left hand, a slight movement in one finger was not sufficient to be counted as a response. After determining the hotspot, the intensity of the stimulation was reduced until the participant's hand responded to only 50% of the pulses delivered to the motor cortex, corresponding to the motor threshold (Verwey et al., 2002). The intensity of stimulation during the rTMS procedure was set at 90% of each participant's individual motor threshold (c.f. Rossi et

al., 2009). Next, the location of the SMA was determined: The vertex (Cz) was measured for each participant and the location of the SMA was defined at 15% of the distance between nasion and inion anterior to Cz on the sagittal midline (Mantovani et al., 2006). After determining the location of stimulation, the TMS coil was positioned over the SMA, at a 45 degree angle.

After the coil was positioned appropriately, 20 minutes of 1 Hz was applied in the experimental group. Participants assigned to the control group were treated as part of one of the experimental conditions, meaning that half of the participants experienced the coil above the SMA and the other half above the PMC region, but they did not receive the actual stimulation. Verwey et al. (2002) found that the rTMS effect is most pronounced after a 20-minute break during which participants could rest. Accordingly, this was applied in the present study.

2.3.3 DSP Test Phase

Participants were again seated in front of the computer to complete the test phase of the DSP task. The test phase consisted of one block with four different manipulations which were counterbalanced across participants. First, in the *familiar* test phase the previously practiced sequences were performed. Second, the *unfamiliar* test phase consisted of new sequences that were not practiced before. Third, participants performed the *single-stimulus* test phase in which they performed the familiar sequences on the basis of the first stimulus. Finally, they executed sequences in the *mixed-familiar* test phase where 75% of the sequences contained two stimuli that were not presented in the practiced order.[3] The familiar and single-stimulus test phases were expected to induce sequencing performance in the chunking mode due to the highly internally driven processing. In contrast, the unfamiliar and mixed-familiar test phases were expected to induce performance in the reaction mode due to the dependency on external stimuli.

The duration of the second day, including both the rTMS treatment and DSP test phase, was about two hours. The total duration of the experiment hence amounted to approximately four hours.

[3] The mixed-familiar test phase is not analyzed in this thesis.

2.4 Data Analysis

The main parameter in this study was the participants' response time (RT) in the DSP task. RTs were calculated as the time that passed between stimulus presentation and depression of the appropriate spatially corresponding key. In addition to RTs, erroneous responses were also taken into account, as well as the amount of correct responses regarding the recall/recognition of their practiced sequence as reported in the awareness questionnaire. In order to analyze performance in the practice phase and test phases, mixed factorial analyses of variance (ANOVA's) were performed.

3. Results

3.1 Practice phase

The development of sequential skill during the practice phase was examined with a 2 (Group: experimental/control) x 8 (Block) x 6 (Key) x 2 (Sequence: 1x6/2x3) repeated measures ANOVA on RTs with Group as a between-subjects variable. Although group treatment did not differ at this point of the experiment, it was included as a variable to ensure that effects found during the test phase could not be traced back to group differences in the practice phase. Indeed, there were no main (p>.95) or interaction effects of Group, ps>.1.

Results revealed effects of Block, $F(7,112)=110.3$, $p<.001$, and Key, $F(5,80) = 109.7$, $p<.001$, indicating that mean RTs reduced with practice and that participants reacted faster past the first key. The latter finding is due to the fact that pressing the first key is always a reaction and therefore holds a special position compared to keys 2-6 which could be anticipated after the display of the first stimulus. Additionally response times on the first key improved less across blocks than keys 2-6, $F(35,560)=10.6$, $p<.001$ (see Figure 2).

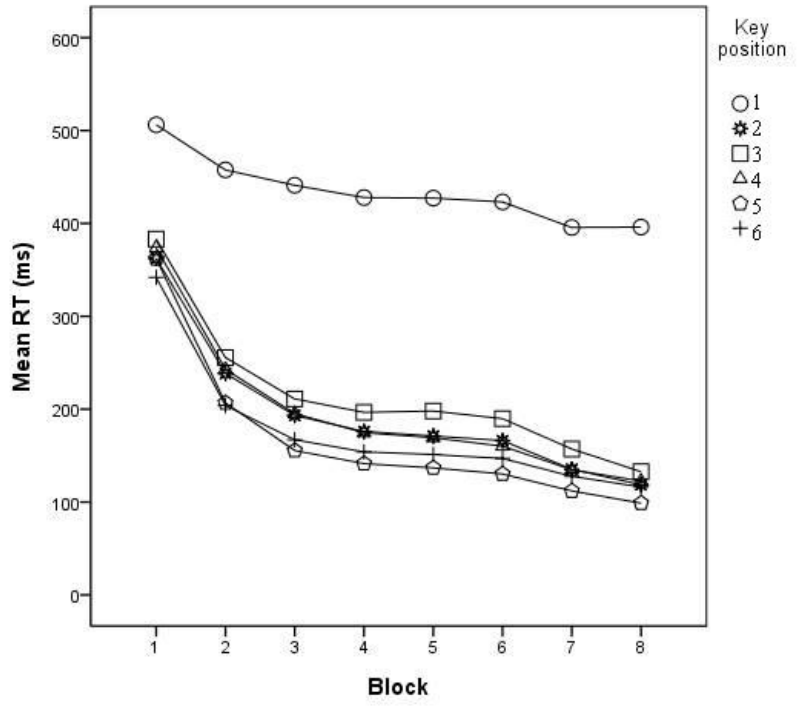

Figure 2. Response times (ms) per key position in the practice block

Furthermore, RTs varied across keys depending on the sequence that was being executed, $F(5,80)=9.5$, $p<.001$. As Figure 3 further illustrates, participants executing the 1x6 sequence displayed a faster reaction time with each key, whereas the 2x3 sequence led to more fluctuations with a particular degraded response time on the third key.

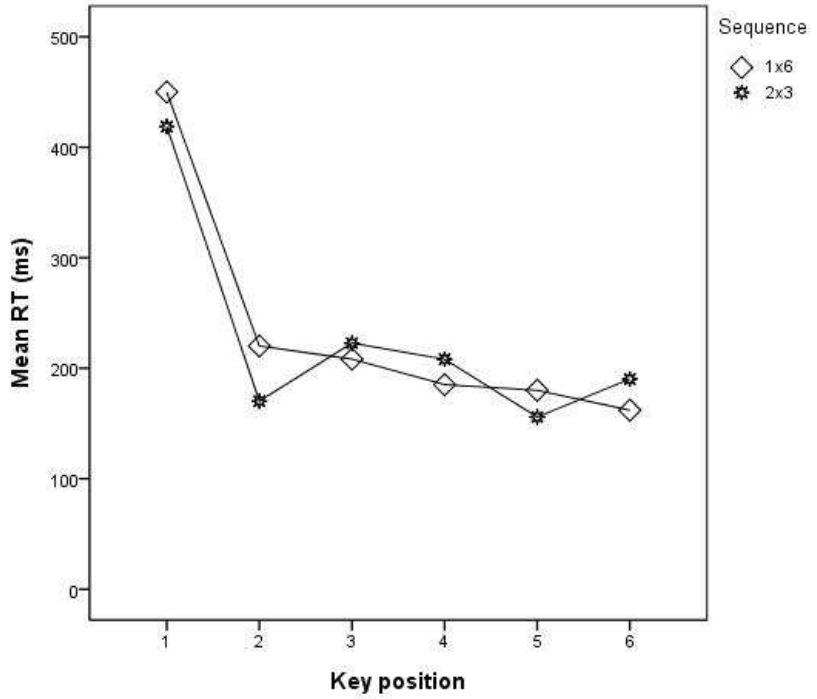

Figure 3. Response times (ms) per 1x6/2x3 sequences across keys

The same ANOVA was performed on error percentages. Results showed that errors declined across keys, $F(5,85)=43$, $p<.001$. However, this is likely the case because the software stopped the sequence after an error occurred. The decline could thus not be caused by an actual accuracy improvement but by the fact that fewer participants actually reached the last key. Furthermore, if it was reached (i.e. if participants did not make mistakes before this key and the sequence was already stopped), the last key was generally executed correctly.

Moreover, an interaction between Block and Key indicated that the amount of errors peaked during the sixth block, except for key 6 where it was continuously low, $F(35,6)=1.7$, $p<.05$. While the accuracy decline during the sixth block (i.e. the last block of the first experimental day) could be attributed to fatigue, the last key could be an exception to this general pattern because it always formed the last action. Participants possibly saw the last key as a reference point that indicated the end point of the sequence. In their sequencing routine they were likely noticing the amount of keys that belonged to each sequence and anticipated its end. In addition, if they reached the last key, they were probably eager to avoid mistakes.

3.2 Test phase

Familiar vs. unfamiliar sequences

Response times during the test phase were analyzed using a 2 (Group) x 2 (Test Phase: Familiar vs. Unfamiliar) x 6 (Key) x 2 (Sequence) repeated measures ANOVA with Group as a between-subjects variable. Results showed that sequences were executed slower in the unfamiliar test phase than in the familiar one (M=438 vs. M=171, respectively), $F(1,17)=1183.2$, $p<.001$. In addition, reactions were again faster past the first key, $F(5,85)=71.6$, $p<.001$. The 1x6 sequence was executed slower than the 2x3 sequence, $F(1,17)=9$, $p<.01$, which is likely due to the higher task difficulty of the 1x6 sequence.

As depicted in Figure 4, participants executing sequences in the familiar test phase showed a clear improvement in RTs past the first key. In contrast, participants during the unfamiliar test phase could not anticipate the forthcoming key and thus stayed at a considerably straight-lined level showing little improvement, $F(5,85)=102.1$, $p<.001$. Similar to the practice phase, there was a Key x Sequence interaction, $F(5,85)=18.4$, $p<.001$. There were no other significant effects, ps>.19.

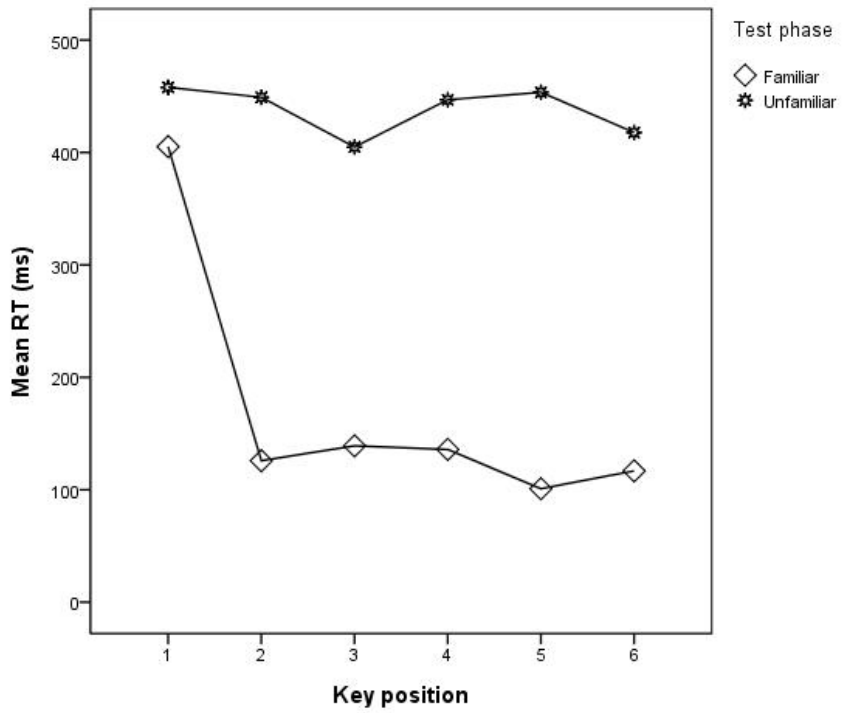

Figure 4. Response times (ms) per familiar/unfamiliar test phase across keys

Results of the same ANOVA performed on error percentages showed that participants made more errors during the unfamiliar sequences than during the familiar sequences (*M*=3.3% vs. *M*=2.5%, respectively), $F(1,17)=8.9$, $p<.01$. Furthermore most errors peaked at key 5, $F(5,85)=4.5$, $p<.001$. Also, during the familiar test phase the 1x6 sequence caused substantially less errors than during the unfamiliar test phase, $F(1,17)=7$, $p<.02$. Although the 2x3 sequence showed less extreme ends, the pattern seems to be reversed, with slightly more errors occurring during the familiar test phase. However, since the error interval taken into account was quite small, we should be careful arriving at conclusions too soon. The Key x Sequence interaction, $F(5,85)=3.6$, $p<.01$ showed a pattern for the 2x3 sequence that consisted of errors steadily increasing until key 5, then dropping to the lowest error rate at key 6. The participants' error rate during the 1x6 sequence however showed more fluctuations, peaking at key 2.

In addition, the experimental group made significantly more errors during the 1x6 sequence than during the 2x3 sequence, whereas the control condition showed the reversed pattern, $F(1,17)=6.4$, $p<.02$. Other main or interaction effects were not found to be significant, *p*s>.1.

Single-stimulus sequences

The RTs of the single-stimulus test phase were analyzed using a 2 (Group) x 6 (Key) x 2 (Sequence) repeated measures ANOVA with Group as a between-subjects variable which again confirmed that key presses were executed faster past the first one, $F(5,85)=16.8$, $p<.001$. There were no other effects, *p*s>.17.

The single-stimulus test phase differed from other phases since it lacked the display of subsequent keys and required participants to retrieve the sequence entirely from memory, except for the first key which was the only cue they received. It is therefore particularly interesting to analyze whether the first key, representing the sequence initiation phase, differed from the remaining keys in terms of RTs. A group difference between experimental and control group could indicate a faster or more effective preparation for the remaining sequence in one group. A one-way ANOVA revealed that the experimental group was indeed slower at responding to the first stimulus than the control group (*M*=479 vs. *M*=400 ms, respectively), $F(1,18)=5.4$, $p<.04$ (see Figure 5). No group differences for the remaining keys were found, *p*s>.24. As further elaborated in the discussion, it is likely that participants in the experimental condition took

considerably longer to retrieve the sequence in the absence of visual cues. TMS has thus possibly temporarily impaired their ability to initiate motor sequences from memory.

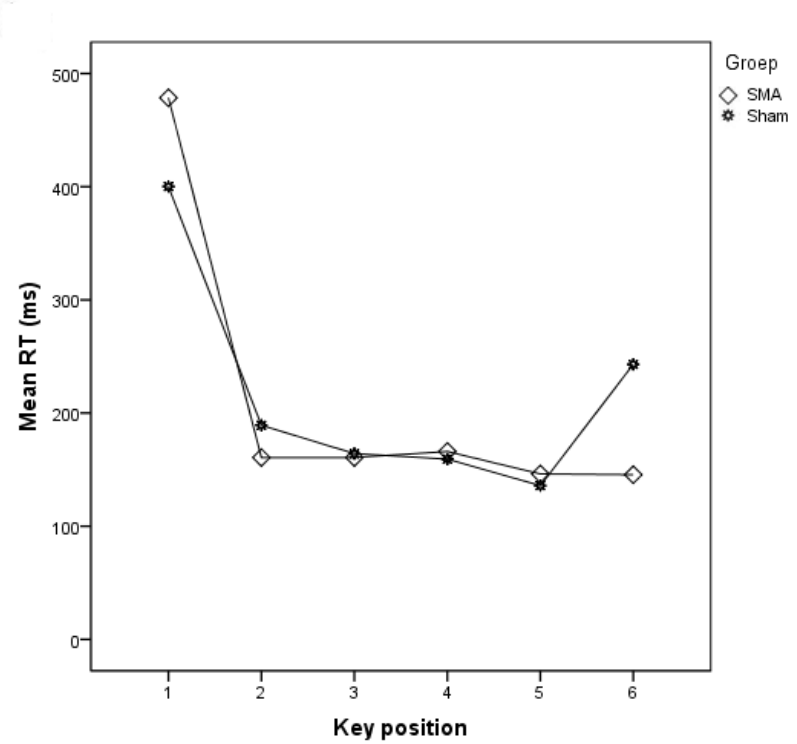

Figure 5. Response times (ms) during the single-stimulus test phase per group across keys

In order to clarify whether there was a difference between experimental and control group regarding errors, we compared the amount of correctly executed sequences in both groups using a one-way between subjects ANOVA. Results showed that there were no group effects, *p*s>.6.

3.3 Awareness

To determine participants' awareness of the sequences we added the number of correctly recalled and recognized sequences, resulting in awareness scores on a scale from 0-4 (see Table 1).

Table 1

Numbers and percentages of participants recalling or recognizing their sequences

Correct	Awareness
0	1 (5%)
1	0 (0%)
2	3 (16%)
3	1 (5%)
4	14 (74%)
Total	19 (100%)

Results of a one-way ANOVA of Awareness and Group showed that both experimental and control group were almost equally aware of their sequences (M=3.5 vs. M=3.4 correct responses, respectively), p>.8. A bivariate correlation revealed that awareness correlated with mean RT during the single-stimulus test phase, $r(17)$=-.64, p<.01, indicating that participants who were more aware of their sequence executed the sequence faster than participants who were less aware. Separate analyses for the experimental and control condition showed that this held true for the control condition, $r(9)$=-.86, p<.01, but not for the experimental condition, p=.48. Awareness thus led to a faster sequence execution only if the participant did not undergo rTMS. If the participant was in the experimental condition awareness did not correlate with a faster execution. It is thus possible that rTMS on the SMA somehow inhibited the ability to use awareness in order to execute the sequences faster. In conclusion, only the control group seemed to benefit from the knowledge about their sequences in terms of increased RTs. Correlations of Awareness and mean RT in familiar or unfamiliar test phases did not reach statistical significance, ps>.18.

4. Discussion

The aim of the present study was to investigate the interaction of cognitive and neurological correlates that are involved in controlling sequential motor skill. Using the DSP task, participants learned two discrete keying sequences until they were thought to perform familiar sequences in the chunking mode. Subsequently, in the experimental group rTMS was applied over the SMA, a structure thought to be involved in the preparation and execution of familiar motor sequences (e.g. Halsband et al., 1994). The SMA was particularly active during a

sequential task that needed to be retrieved from memory rather than being responded upon sensory cues (Kennerley et al., 2002). After the stimulation we introduced previously practiced (familiar) and unpracticed (unfamiliar) test phases as well as a single-stimulus test phase where only the first key was displayed and participants had to complete the remaining sequence entirely from memory. During both the familiar and single-stimulus test phases participants were expected to execute sequences in the chunking mode, in which performance is based on internal representations and does not necessarily require external guidance. We expected a performance decline during both familiar and single-stimulus test phases after the induction of rTMS over the SMA because the ability of the SMA to help them initiate the chunking mode during familiar sequences would be reduced.

Results showed that sequences in the unfamiliar test phase were performed slower than sequences in both familiar and single-stimulus phases. During the execution of unfamiliar sequences, participants could not anticipate the sequence yet and needed to respond to each cue individually, which took considerably more time than executing familiar sequences. Participants thus likely executed sequences in the reaction mode when they experienced unfamiliar sequences (Verwey & Abrahamse, 2012). During the familiar sequence however, they were able to execute the same sequences they had learned during the practice phase. Here, they could react much faster because they knew which sequence to expect and could respond accordingly. In line with the DPM, loading the motor buffer with a chunk apparently took considerably less time than selecting individual sequence elements that needed to be loaded one by one. Accordingly, the emergence of motor chunks during the chunking mode caused a decreased involvement of the cognitive processor. The motor processor could therefore execute the sequences faster than if both systems operated in parallel (Verwey, 2001).

In order to clarify whether the SMA is involved in the execution of sequential action in the chunking mode, we analyzed whether there was a group difference in RTs in the test phases thought to induce the chunking mode (i.e. familiar and single-stimulus test phase). The hypothesis that the experimental group showed a slowed performance compared to the control group during familiar and single-stimulus test phases could not be confirmed. The lack of a difference between both groups does not support the idea that the SMA is involved in the execution of sequences performed in the chunking mode.

According to Verwey (2010), besides sequence execution there is also a processing phase classified as sequence initiation, reflected in RTs on the first key. Since previous studies pointed to the SMA as being involved in sequence preparation, it was hypothesized that the experimental groups showed a delayed response on the first key during both familiar and single-stimulus test phases (e.g. Roland et al., 1980; Thaler et al., 1988; Deiber et al., 1996; Graybiel, 2008). Interestingly, results showed that during the single-stimulus test phase, groups differed in RTs on the first key. Apparently the experimental group took considerably longer to retrieve sequences from memory. This supports our hypothesis that the SMA seems to be responsible for initiating sequences performed in the chunking mode.

Usually, in movement sequences without external guidance, the SMA identifies sequential elements and transmits them to the primary motor cortex (M1). M1 is then responsible for immediately executing each element (Verwey et al., 2001). The SMA could be involved in both the identification of sequential elements as well as the transmission to the M1, which were impaired by rTMS, as reflected in slowed RTs of the first key of the experimental group. Accordingly, M1 could not execute the motor plans of the SMA as quickly as usual (i.e. as in the control group) because the SMA did not provide the necessary planning information on time. One can also assume that rTMS caused a translational problem between cognitive and motor processor. The cognitive processor is mostly engaged in preparing familiar sequences, whereas the motor processor is responsible for executing them. Usually the cognitive processor triggers the motor processor and thereby initiates the next motor chunk (Verwey et al., 2010). In the present study however, this transmission was possibly impaired by rTMS on the SMA. SMA could thus be responsible for translating sequential information to M1.

However, the finding that the SMA is responsible for preparing sequences performed in the chunking mode could not be confirmed for the familiar test phase, in which no group differences in sequence initiation were found. It is possible that participants employed different strategies for familiar and single-stimulus test phases because they could anticipate the nature of the following task, as specific instructions were given prior to each test phase. They possibly expected the single-stimulus phase to be more difficult because they had to rely on their working memory to execute keys 2-6. Subsequently they possibly took more time to prepare these sequences as compared to familiar sequences, where they knew that in case of doubt they could always fall back on the visual cue. Since the task difficulty could have been perceived as higher

than during the familiar test phase, the demands on the cognitive processor could have been higher as well. It is possible that the cognitive processor took more effort to prepare the sequences because it could not fall back on visual cues and thus prepared the sequences more thoroughly which took more time. The SMA could thus particularly be responsible for preparing sequences performed in the chunking mode, especially for tasks that only provide the first cue. Preparation hence seems to be a crucial element for the execution of sequences in the chunking mode. Confirming this observation, there were no effects on key 1 during the unfamiliar test phase, indicating that preparation is a key process for the chunking but not for the reaction mode. Preparation during the reaction mode cannot take place, because participants cannot anticipate the forthcoming stimulus.

Regarding differences in task difficulty, results showed that during the familiar test phase the experimental group made more errors during the 1x6 sequence than during the 2x3 sequence, pointing to the fact that the SMA could be more engaged in executing complex sequences. This is in line with previous findings, e.g. Rao et al. (1993) who demonstrated that the complexity of sequential finger movements positively correlated with SMA activity. More difficult tasks thus led to a higher SMA activity as compared to simple movements which elicited less activity. Gerloff et al. (1997) also stated that the SMA is involved in organizing forthcoming movements in complex motor sequences as well as planning future elements. In contrast, this finding does not support the aforementioned results found in the present study that proposed a particular role for the SMA during preparation but not execution of sequential movement. SMA may thus have been responsible for both the accurate execution of complex sequences (i.e. 1x6) during the familiar test phase and the initiation of sequences in the single-stimulus test phase.

Furthermore, it was found that the 1x6 sequence was generally executed more slowly than the 2x3 sequence. This finding was expected due to the increased task difficulty that the 1x6 sequence posed. However, instead of decreasing after the third key, RTs of the 2x3 sequences decreased after the second key. This could be traced back to individual differences in chunking patterns. Whereas some participants may have remembered the sequence as a 1x3 plus 1x3 sequence, others could memorize it as a 1x2 plus 1x4 sequence. In order to avoid a range of chunking strategies one could have programmed a short break between the two 1x3 parts to encourage participants to chunk in a certain manner. However, in the present study chunking was

not imposed because the focus initially did not lie on the distinction between 1x6 and 2x3 sequences.

Analysis of the awareness questionnaire showed that both groups were almost equally aware of the sequences. While in the control group explicit knowledge led to a faster sequence execution, it did not affect performance in the experimental group. It thus seems that only participants whose SMA was unaffected (i.e. control group) could benefit from explicit sequence knowledge. This indicates that the SMA may somehow be responsible for utilizing explicit knowledge of sequences towards improved performance, which was prevented by rTMS.

Limitations of the present study mostly relate to the fact that rTMS alone cannot provide a precise localization of the targeted neural structure. Since we identified the location of the SMA manually it is possible that the measurement was not accurate and that we did not locate the SMA but some neighboring structures, like the pre-SMA. This could explain why the results are mostly related to preparatory activities instead of internal features, since the pre-SMA is also highly involved in preparatory actions (Graybiel, 2008). A solution to prevent inaccuracy would be a combination of rTMS and fMRI. Since fMRI is more accurate than rTMS and can precisely locate the SMA, there would be no doubt if the rTMS is really applied to the correct area.

Additionally, some participants reported that, although they were classified as being right-handed according to Annett's Handedness Inventory (1970), they were also trained using their left hand due to various reasons such as playing the piano or gaming. It is possible that those participants differed from others because their brains might have reorganized the cortical maps of their left hand if their skill level was advanced enough. Landau and D'Esposito (2006) examined the influence of long-term motor expertise in pianists and nonpianists and found that pianists developed faster RTs and superior sequence acquisition as compared to nonpianists. Accordingly their motor processor may be involved more quickly because they might learn the sequences faster. Repetitive TMS could thus have influenced their cortical structures in a different way. It would be interesting to see how these left-handed skills relate to sequence execution by including that question in the handedness or awareness questionnaire.

Furthermore, participants knew which test phase to anticipate because the instructions stated the upcoming test phase. They knew that for example during the familiar test phase they could still fall back on sensory cues, whereas during the single-stimulus test phase they knew they had to retrieve the sequences from memory and could hence take preparative actions. The

SMA could thus have prepared for internal memory retrieval. It could be useful to analyze whether this information influences neural preparatory actions. Previous studies showed that response labels used in the instruction directly determined what is learned in sequence learning (Wenke & Frensch, 2005; Gaschler, Frensch, Cohen, & Wenke, 2012). Following studies could take that knowledge into account and could create three groups: group one with the same instructions as in the present study, that is, correctly stating which sequence to expect, group two that does not receive any instructions, and group three that gets wrong instructions about upcoming sequences that do not match their expectations. It is possible that concealing information about the sequences would lead to a more spontaneous reaction, as participants then cannot form a strategy that prepares them for the upcoming response.

Finally, it is difficult to draw conclusions yet because as mentioned before, this study is part of a bigger study. In the present study we only analyzed a subset of the whole experiment whereas a higher sample-size after the completion of the bigger study could lead to different conclusions. For instance, at face value there is also a difference in RTs between the first key and the remaining keys of the familiar test phase. However, this difference is not significant yet. More participants could thus yield clearer results that could explain more of the findings we came across in this study.

After the complete study is finished one could also speculate about applications of these findings. If it held true that the SMA was indeed involved in preparatory actions of sequences, one could use rTMS to excite the SMA of people who have trouble initiating movements, such as Parkinson's disease patients. Parkinson's disease especially disrupts basal ganglia function. However, since basal ganglia and SMA are connected via the thalamo-cortical loop and SMA input arises largely from the basal ganglia, SMA function is also impaired in Parkinson's disease (Tokuno et al, 1992). In addition, Cunnington et al. (1995) found that particularly pre-movement SMA activity is disturbed in patients with Parkinson's disease. Cunnington et al. (1996) found evidence that patients with Parkinson's disease also show a functional impairment in the SMA that can be observed in a performance decline during sequential movements. They arrived at this conclusion by applying TMS over the SMA, which disrupted the preparation of motor sequences, similarly to the present study. Instead of using rTMS to inhibit cortical function, future research could thus employ this technique in order to excite the SMA of people with Parkinson's disease or other related movement disorders. Moreover, the people with less severe disorders who have

trouble preparing movements could make use of this technique. It is often observed that elderly people are poor at handling complex sequences (e.g. driving a car) which could be due to a disintegrated preparation. Repetitive TMS could excite their SMA, which could enable them to prepare their upcoming actions properly. However, since the effects of rTMS are quite short-lasting not to mention expensive, it is not realistic to apply it to a larger audience. Instead, research could find other ways to excite the SMA for longer periods, for example by means of a cortical implant or deep brain stimulation, which is currently applied for patients with Parkinson's disease or Tourette's syndrome (Moro & Lang, 2006).

Further research could also combine rTMS with electroencephalography (EEG) in order to measure whether rTMS to the SMA really impaired the preparation of sequences, or if the group differences were caused by other factors. EEG has been proven to be a suitable technique to measure preparation of sequence production (Van der Lubbe, et al., 2000; De Kleine & Van der Lubbe, 2011). EEG is especially useful for this follow-up study because, unlike CT or MRI scans, it can provide a millisecond-range temporal resolution despite its limited spatial resolution. Event-related potentials (ERPs) are suitable to track the functional processes underlying movement over time. If ERPs showed a significantly higher increase of neural activity for the experimental group before or during the first key, this would support our findings. It would confirm that the SMA is engaged in preparatory effects of sequential movement. In the present study we found differences between the familiar and the single-stimulus test phase regarding preparation. It would be interesting to measure ERPs during both familiar and single-stimulus phases. Differences in ERPs during EEG could then mean that although familiar and single-stimulus test phases both induce the chunking mode, there might be differences between those two "chunking" modes with regards to the presence of visual cues. There could be another mechanism that cannot be called chunking or that is part of a subcategory of chunking. The ideal study would thus be a combination of rTMS, fMRI and EEG.

In conclusion, results offered partial support for the notion that the SMA is involved in sequence execution in the chunking mode. Specifically, this study contributed to further unraveling the role of the SMA during sequential action by showing that it is likely involved in preparatory processes of sequences. The precise role of the SMA should be further investigated by future research.

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