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Cognitive and neural foundations of discrete sequence skill: A TMS study

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ABSTRACT

Executing discrete movement sequences typically involves a shift with practice from a relatively slow, stimulus-based mode to a fast mode in which performance is based on retrieving and executing entire motor chunks. The dual processor model explains the performance of (skilled) discrete key-press sequences in terms of an interplay between a cognitive processor and a motor system. In the present study, we tested and confirmed the core assumptions of this model at the behavioral level. In addition, we explored the involvement of the pre-supplementary motor area (pre-SMA) in discrete sequence skill by applying inhibitory 20 min 1-Hz off-line repetitive transcranial magnetic stimulation (rTMS). Based on previous work, we predicted pre-SMA involvement in the selection/initiation of motor chunks, and this was confirmed by our results. The pre-SMA was further observed to be more involved in more complex than in simpler sequences, while no evidence was found for pre-SMA involvement in direct stimulus–response translations or associative learning processes. In conclusion, support is provided for the dual processor model, and for pre-SMA involvement in the initiation of motor chunks.

1. Introduction

Most of the complex motor actions that people perform in daily life consist of series of relatively simple movements that are executed in a specific order. In this respect one may think of actions such as playing the piano and lacing a shoe. With practice, the order of movements is learned and the action gradually becomes automated in the sense that little attentional monitoring is needed for proper execution. In short, a motor skill has developed. Over the last decades, ample research has been devoted to unraveling the cognitive and neural mechanisms underlying sequential motor skill (e.g., Abrahamse, Ruitenberg, De Kleine, and Verwey (2013), Ashby, Turner, and Horvitz (2010), Keele, Ivry, Mayr, Hazeltine, and Heuer (2003), Penhune and Steele (2012), Verwey (2001), Wymbs, Bassett, Mucha, Porter, and Grafton (2012)). In the current study, we further explore the cognitive and neural substrates that underlie the execution of well-practiced discrete movement sequences. Below, we first discuss a recent framework that we proposed for understanding the production of such sequences—the so-called dual processor model (DPM; Abrahamse et al. (2013), Verwey (2001)). Briefly, this model builds on the interplay between a cognitive processor and a motor system, and it brings together a number of features that are reminiscent of other frameworks of sequence skill (e.g., Abrahamse, Jimenez, Verwey, and Clegg (2010), Hikosaka et al. (1999), Keele et al. (2003), Klapp (1995, 2003), Rosenbaum, Kenny, and Derr (1983)). Here we do not elaborate on these other frameworks as these and their link to the DPM are described in detail elsewhere (Abrahamse et al., 2013). Rather, we zoom in on the cognitive processor and its various features, and report an experiment that tests the involvement of the pre-supplementary motor area (pre-SMA) with respect to these features.

1.1. Discrete sequencing skill

The DPM describes a cognitive architecture underlying the production of discrete movement sequences that involves a cognitive processor and a motor system (Abrahamse et al., 2013; Verwey, 2001). This framework has mainly been derived from work with the so-called discrete sequence production (DSP) task (Verwey, 1999). In the standard version of this task, participants respond to fixed series of two to seven stimuli by means of spatially compatible key presses, and accordingly learn to perform fixed movement (i.e., response) patterns. Participants initially respond to each individual stimulus as they lack internal representations of the sequence. In this so-called *reaction mode* the cognitive processor is assumed to be responsible for the

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translation of each stimulus into the appropriate response, which is subsequently carried out by the motor system.

With practice, the movement sequences are learned so that participants can identify the to-be-executed sequence on the basis of just the first sequence-specific stimulus, and can execute the whole sequence at high rate without the need for full processing of the ensuing stimuli. This performance improvement is attributed to the development and use of *motor chunks*: representations of successive responses (here: key presses) that can be prepared and executed as if these response series are a single response (e.g., Verwey (1999)). At this stage, the cognitive processor can select such an entire motor chunk on the basis of just a single stimulus. It then loads the motor chunk into a motor buffer, after which the motor system executes the elements represented within the motor chunk by reading them from the motor buffer. With longer sequences, participants spontaneously segment their sequence into multiple motor chunks that are concatenated during execution (Bo & Seidler, 2009; Kennerley, Sakai, & Rushworth, 2004; Ruitenberg, Abrahamse, De Kleine, & Verwey, 2012; Sakai, Kitaguchi, & Hikosaka, 2003; Verwey, Abrahamse, & Jiménez, 2009; Verwey & Eikelboom, 2003). Executing sequences through one or more motor chunks is referred to as the *chunking mode*.

The DPM additionally postulates that, after loading motor chunks into the motor buffer, the cognitive processor can assist the motor processor in executing the elements within these chunks by engaging in direct stimulus–response (S–R) translations (Verwey, Abrahamse, & De Kleine, 2010; Verwey, Abrahamse, De Kleine, & Ruitenberg, 2014). Consequently, two processes for response generation can occur simultaneously in the chunking mode: the motor system triggers responses by reading response-related codes from the motor buffer, and the cognitive processor selects responses on the basis of S–R translations. This race between these two response generation processes results in the fastest possible responses (i.e., statistical facilitation; Verwey (2001, 2003)).

Besides the reaction and the chunking mode, the DPM distinguishes a third, *associative mode* (cf. Verwey & Abrahamse, 2012). In this mode, performance is still based on – and thus requires – external stimuli that are one by one translated into the correct response by the cognitive processor. However, this translation process is facilitated by sequential learning in the sense that associations between successive S–R events prime forthcoming events. The associative mode comes into play when sequential knowledge exists but is either too weak (e.g., with limited practice) or insufficient (e.g., when events occasionally do not follow the learned sequential order; cf. Verwey and Abrahamse (2012)) to fully drive performance. In contrast to the chunking mode, the associative mode is characterized by performance improvements that are relatively small (i.e., average response times do not typically drop below 250 ms), by more or less equally divided response times across all key-presses, and by a lasting dependence on stimulus presentation.

The cognitive processor thus performs various roles across the three modes. In recent years its precise nature is beginning to be unraveled (see Abrahamse et al. (2013)). For example, Verwey et al. (2010, 2014)) demonstrated that the resources of the cognitive processor can be distributed flexibly across several tasks. That is, when combined with a secondary tone counting task, it was observed that performance in terms of response times on the (primary) sequencing task was slightly – but not severely – impaired for a few key-presses after the presentation of a single tone. This was interpreted as support for a domain-general resource: the cognitive processor can briefly interrupt its contribution to ongoing sequencing performance (by racing with the motor system) to manage the demands of the tone counting task, after which (if the sequence is still ongoing) it can switch back to racing with the motor system. Moreover, a number of studies indicate that performance of the cognitive processor can be moderated by the perceptual or task context (Ruitenberg et al., 2012; Ruitenberg, De Kleine, Van der Lubbe, Verwey, & Abrahamse, 2012). However, much less attention has hitherto been given to the neural underpinnings of this cognitive processor.

1.2. Neural substrate of the cognitive processor

Over the last two decades an increasing number of studies have explored the neural underpinnings of sequential action in general, using techniques such as positron emission tomography (PET; e.g., Jenkins, Brooks, Nixon, Frackowiak, and Passingham (1994), Jenkins, Jahanshahi, Jueptner, Passingham, and Brooks (2000)), functional magnetic resonance imaging (fMRI; e.g., Toni, Krams, Turner, and Passingham (1998), Wymbs et al. (2012)) and transcranial magnetic stimulation (TMS; Kennerley et al. (2004), Verwey, Lammens, and Van Honk (2002)). The studies have shown that various brain structures including (pre-)motor, prefrontal, and parietal cortices, the basal ganglia, and the cerebellum can form networks that are involved in sequencing skill. However, it remains unclear what functions are related to each specific area and/or corresponding network. In addition to these brain areas, previous studies suggest the involvement of the pre-SMA in the production of movement sequences (e.g., Kennerly et al. (2004), Picard and Strick (2001), Wymbs and Grafton (2013)). In the present study, we specifically explore its potential involvement in the various roles assigned to the DPM's cognitive processor—that is, motor chunk initiation, (online) S–R translations, and association-based priming of responses.

A priori, there are strong arguments to expect a substantial contribution of the pre-SMA to some of the functions that we have attributed to the DPM's cognitive processor. The pre-SMA is associated with cognitive aspects across a variety of paradigms (for an overview see Picard and Strick (2001)) and is anatomically connected to pre-frontal areas that are known to be involved in higher cognitive (i.e., executive) functions (Luppino, Matelli, Camarda, & Rizzolatti, 1993; Picard & Strick, 1996; Wang, Isoda, Matsuzaka, Shima, & Tanji, 2005). This directly distinguishes the pre-SMA from the more posterior located SMA (or SMA-proper), which both functionally and anatomically can be assumed to be more motor oriented (He, Dum, & Strick, 1995; Luppino et al., 1993; Picard & Strick, 1996, 2001). These differences in connectivity between SMA and pre-SMA suggest that they have distinct roles in sequential motor control. Kennerley et al. (2004) and Verwey et al. (2002) employed repetitive TMS (rTMS) to explore the contribution to sequencing skill of the pre-SMA and SMA, respectively, and indeed reported distinct roles for each. Specifically, whereas Verwey et al. (2002) observed that all elements within a well-learned DSP sequence were equally slowed following rTMS stimulation of the SMA, Kennerley et al. (2004; Exp. 2) found that rTMS stimulation of the pre-SMA slowed only those key presses that reflected initiation of a motor chunk within an ongoing sequence. In terms of the DPM, these findings suggest that the SMA is probably responsible for loading and/or executing individual sequence elements (cf. motor system), while the pre-SMA may be responsible for selecting entire motor chunks from memory and/or loading these chunks into the motor buffer (cf. cognitive processor).

Interestingly, while Kennerley and colleagues reported slowed motor chunk initiation *within* an ongoing sequence in their Experiment 2, they did *not* observe any effects on sequence initiation. This goes against the assumption of the DPM that motor chunk initiation in general – independent of whether the motor chunk entails the start of the sequence or not – is a dedicated process driven by the cognitive processor. However, participants in

Experiment 2 of the study by Kennerley et al. (2004) were only briefly trained on a single, 12-element keying sequence, while the DPM builds on DSP studies that employed two extensively practiced and shorter (mostly 6-element) sequences. Especially the use of only a single sequence may be hypothesized to account for the absence of effects on sequence initiation in the study of Kennerley et al. (2004), as this may have allowed for preparation of the first motor chunk of the sequence to occur mostly before the first stimulus appeared so that initiation processes were no longer reflected in the first response time of the sequence. In contrast, when two sequences are executed in a random order, sequence selection can only commence after presentation of the first key-specific stimulus.

Indeed, when in two subsequent experiments participants performed two sequences, Kennerley et al. (2004; Exp. 3 and 4) observed that sequence initiation was slowed after applying rTMS stimulation to the pre-SMA. However, these latter experiments did not examine the possibility that the sequences were segmented into multiple motor chunks, and consequently did not differentiate between the effects of rTMS stimulation of the pre-SMA on initiation of the first motor chunk of a sequence (i.e., sequence initiation) and initiation of subsequent motor chunks. Here, we therefore set out to test the prediction that rTMS stimulation of the pre-SMA should similarly affect both sequence initiation and subsequent motor chunk initiation when employing the typical DSP design, in which sequences have often been observed to be segmented into several motor chunks (e.g., De Kleine and Verwey (2009a, 2009b), Ruitenberg, Abrahamse, and Verwey (2013), Verwey et al. (2009), Verwey and Eikelboom (2003)). This also provides a test on whether the pre-SMA remains involved after more extensive practice than in the study by Kennerley et al. (2004).

Finally, various studies have observed larger pre-SMA activity in relatively complex than in simpler sequences (Boecker et al., 1998; Gerloff, Corwell, Chen, Hallett, & Chen, 1997; Grafton, Hazeltine, & Ivry, 1998). The presumed efforts related to such sequence complexity could be speculated to differentially tax the cognitive processor from the notion that the latter is a graded resource (cf. Verwey et al., 2014), and this thus tentatively links the pre-SMA to the cognitive processor, too.

1.3. The present study

We employed the DSP task to study the involvement of the pre-SMA in the various functions assigned to the DPM's cognitive processor. Participants extensively practiced two discrete keying sequences across eight practice blocks. The two sequences were then performed in four test conditions. In the *familiar condition* and the *single-stimulus condition*, participants performed their learned sequences in the chunking mode. In the familiar condition, participants performed the sequences on the basis of key-specific stimuli, like during the practice phase. In the single-stimulus condition only the first key-specific stimulus of each sequence was displayed and participants completed the rest of the sequence from memory. In the *mixed-familiar condition*, participants performed their learned sequences, but in the majority of the sequences the order of key-specific stimuli deviated slightly from what had been learned during the practice phase. This was expected to trigger sequence execution in the associative mode (Verwey & Abrahamse, 2012). Finally, in the *mixed-unfamiliar condition*, participants performed novel sequences (most also including a deviation from the base sequence) so that the sequences are executed in the reaction mode.

At the behavioral level, these test conditions enabled exploration of the core functions of the cognitive processor as proposed by the DPM (Abrahamse et al., 2013). First, we expected to replicate the three-mode division (reaction, associative and chunking modes), which has so far only been shown once (Verwey & Abrahamse, 2012). Specifically, sequences should be performed fastest in the familiar and single-stimulus test conditions, as these allow for execution in the chunking mode, and sequences should be performed slightly faster in the mixed-familiar test condition (assessed only on the sequences without deviants, see below) than in the mixed-unfamiliar test condition due to priming on the basis of sequence knowledge. Additionally, in line with the notion that the cognitive processor races with the motor system on the basis of direct S–R translation, performance was predicted to be somewhat slower in the single-stimulus than the familiar test condition as the absence of stimuli prevents such racing. These predictions at the behavioral level, however, only provide the necessary conditions to explore the involvement of pre-SMA in the functions of the DPM's cognitive processor.

We applied off-line low-frequency (1 Hz) subthreshold rTMS stimulation to either the pre-SMA or one of two control conditions (between-subject) for a period of 20 min. It has been established that such low-frequency rTMS stimulation has inhibitory effects on the stimulated region that outlasts the stimulation period (cf. Chen et al., 1997; Wassermann et al., 1996; see also Kennerley et al., 2004; Rossi & Rossini, 2004; Verwey et al., 2002). The involvement of the pre-SMA in a particular process can thus be inferred from the slowing of the responses due to the inhibitory effects of rTMS. Specifically, we explored pre-SMA involvement across the functions of the DPM's cognitive processor.

We hypothesized that (I) when sequencing performance is based on internal sequence representations – i.e., motor chunks in the chunking mode – the pre-SMA is involved in the selection and initiation of these motor chunks. This would be indicated by slowed performance in the single-stimulus and familiar conditions of the first key-press of any motor chunk (both the first motor chunk of a sequence, and motor chunks that are started somewhere half-way through an ongoing sequence). By using two randomly presented sequences (rendering it impossible to select motor chunks before presentation of the first stimulus), we aimed to show that the pre-SMA is involved not only when selecting the 'next' motor chunk within an ongoing sequence, but also when initiating the first motor chunk of that sequence.

Additionally, we explored (II) if the pre-SMA is differentially involved in sequences with different levels of complexity (cf. Boecker et al., 1998; Gerloff et al., 1997; Grafton et al., 1998). Finally, we post-hoc explored pre-SMA involvement across the other functions that we have assigned to the cognitive processor. Specifically, these are (III) direct S–R translation that is assumed to take place with sequence execution in the reaction mode, (IV) association-based priming that is assumed to underlie the associative mode (by zooming in on performance in the sequences without deviants in the mixed-familiar condition), and (V) direct S–R translation that underlies the race between the cognitive processor and the motor system in the chunking mode (by zooming in on the expected performance difference between sequences in the familiar and single-stimulus conditions).

2. Method

2.1. Participants

Forty-eight healthy students from the University of Twente participated in the study (10 male, 38 female). They were aged between 18 and 28 years ($M=21$, $SD=2$). All participants were classified as being right-handed according to Annett's (1970) Handedness Inventory 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, psychiatric, or hearing disorders, any medical conditions, pacemaker or other metals located near the head, pregnancy,

alcohol/drug consumption 48 h/2 months prior to the experiment, and smoking history (cf. Rossi, Hallet, Rossini, & Pascual-Leone, 2009). Written informed consent was obtained from all participants, who could receive credits they needed as part of a course requirement. The study was approved by the Medical Ethical Committee of the Medical Spectrum Twente (MST), Enschede, The Netherlands.

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 Pentium© IV Windows XP© PC. Windows services that could affect reaction time measurements were shut down. Stimuli were presented on a 17-inch Philips 107 T5 CRT display. Responses were given on a standard qwerty-keyboard.

Transcranial magnetic stimulation was delivered using a high power Magstim Rapid 2 Stimulator© (The Magstim Company, Whitland, UK), 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© and automatically corrected for minor head movements made by the participants, to ensure that the coil remained positioned above the target area during stimulation.

2.3. Discrete sequence production task

Participants were instructed to place the little, ring, middle and index fingers of their left hand on the *c*, *v*, *b* and *n* keys, respectively. Four horizontally aligned black square placeholders were presented on a computer display with a white background (see Fig. 1), and these stimulus squares spatially corresponded with the alignment of the four response keys. As soon as one of the placeholders was filled with a green color, participants pressed the corresponding key (e.g., *c*, for the leftmost square). When the correct response was given the color in the square changed back to the white background color for 50 ms[1], after which the next stimulus of the sequence was presented. Once all stimuli of the sequence were presented and correctly responded to, the display was erased white for 1000 ms to indicate completion of the sequence. The placeholders were then presented again for 1000 ms before the first stimulus of the next sequence was displayed. Participants were instructed to respond as fast and accurately as possible. An incorrect response resulted in an error message that was presented for 2000 ms. This relatively long presentation time was used to motivate participants to prevent errors. The ongoing sequence was then aborted and followed by a 1000 ms white screen, after which the placeholders were presented for 1000 ms and the next sequence started.

In the practice phase, participants responded to two series of six stimuli, yielding two 6-key response sequences. In order to prevent finger-specific effects on individual response times, response keys were rotated across sequential positions across all participants. This resulted in four versions of each sequence, namely *ncbncb*, *cvncvn*, *vbcvbc* and *bnvbnv* (the 2 × 3 sequence) and *nvbcbv*, *cbnvnb*, *vncbcn* and *bcvnvc* (the 1 × 6 sequence, cf. Verwey et al. (2002)). The two sequences that a participant practiced never started with the same key press and were presented in random order. Each practice block included 90 trials per sequence. With six practice blocks on the first day of the experiment and two practice blocks on the second day, participants completed 720 trials for each sequence. There was a short 40 s break halfway through each practice block and a 4 min break at the end of each practice block. Before each break, participants received feedback on their mean response time and error percentage.

The test phase included four blocks of 60 trials, each block including a different experimental condition. The blocks were separated by 40 s breaks and the order of the four blocks was counterbalanced across the participants. Two test blocks involved the two sequences that participants had performed during the practice phase. In the *familiar* condition participants responded to the same order of key-specific stimuli as during the practice phase. In the *single-stimulus* condition, participants performed their practiced sequences on the basis of only the first sequence-specific stimulus. After presentation of that stimulus, the placeholders remained white and participants had to complete the sequences from memory. In the *mixed-familiar* condition the practiced sequences were carried out, but in 75% of the sequences two of the stimuli at the sequential positions 2–6 were randomly changed. These two deviants never occurred at successive positions, resulting in sequences with deviants at positions 2 and 4, 2 and 5, 2 and 6, 3 and 6, and 4 and 6 (indicated as [24], [25], etc.). The remaining 25% of the sequences did not involve such deviants (indicated as [00]) and thus were the sequences that participants had learned during the practice phase. Finally, the *mixed-unfamiliar* condition involved two unfamiliar 6-key sequences that were also taken from the eight versions of the 6-key sequences developed by rotating keys across sequential positions. In this condition, too, 75% of the sequences involved two random deviations from the unfamiliar base sequences. Each test block was followed by presentation of feedback regarding mean response time and error percentage.

[1] The response-to-stimulus interval (RSI) of 50 ms differs from that in a typical DSP task, in which an RSI of 0 ms is employed. The here employed RSI allowed participants to perceive an occasional repetition of the same stimulus location in case of random deviants in the mixed-familiar and- unfamiliar conditions of the test phase (cf. Verwey & Abrahamse, 2012).

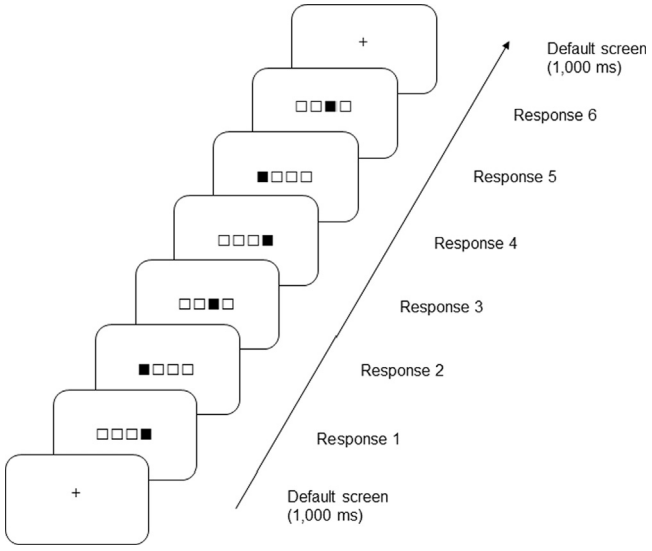

Fig. 1. The DSP task. Stimulus presentation in the DSP task involves the display of four stimulus placeholders that spatially correspond to four responses keys. When one of the placeholders is filled, participants respond by pressing the corresponding key press. Please note that we adopted a response-to-stimulus interval of 50 ms, which however is not depicted in the figure.

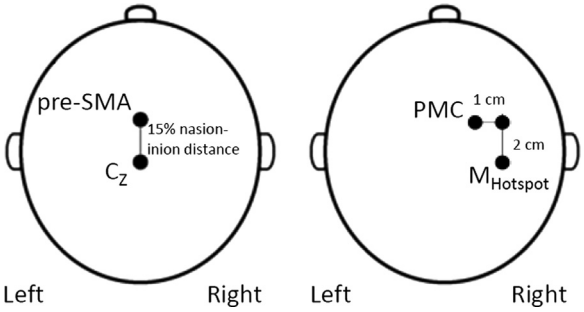

Fig. 2. Stimulation sites. Localization of the stimulation site for 1 Hz rTMS of the pre-SMA and PMC.

2.4. Repetitive TMS

After the final practice block on the second day of the experiment, participants were seated in a dental chair that allowed them to sit in a comfortable position for the duration of the rTMS procedure. Participants were randomly assigned to one of three rTMS groups. We opted for a between-subject rTMS design to avoid cross-over of learning effects between the various rTMS conditions. For each participant we first determined the location of the motor hotspot (i.e., the location on the right primary motor cortex (M1) that evoked 100% responsitivity in the participant's left hand) as well as the motor threshold (MT; i.e., the magnetic intensity at which the hand or thumb responds – as witnessed by visual inspection – in 50% of the stimulations to the hand area of the motor cortex; e.g., Schutter and Van Honk (2006), Verwey et al. (2002)). We used the ascending staircase method described by Schutter and van Honk (2006). First, the M1 of the participant was stimulated with single-pulse TMS at a low intensity. The intensity was slowly increased until visible responses of the hand or thumb appeared. Second, the intensity was set so that responsitivity was 100% (i.e., each pulse elicited a visible response). Third, the intensity was optimized so that it evoked responses during 50% of the pulses; this intensity is the MT of the individual participant (M MT=60% of maximum 1.5 T stimulator output, SD=8%).

Next, the stimulation site was determined for each participant (see Fig. 2). The pre-SMA was assumed to be at 15% of the distance between nasion and inion anterior to Cz on the sagittal midline (cf. Mantovani, Simpson, Fallon, Rossi, & Lisanby, 2010). Hence, we stimulate a more anterior location in comparison to the rTMS-SMA study by Verwey et al. (2002), who targeted the SMA at 10% of the distance between nasion and inion anterior to Cz on the sagittal midline. Across participants in the pre-SMA group, this procedure yielded a mean coil placement at

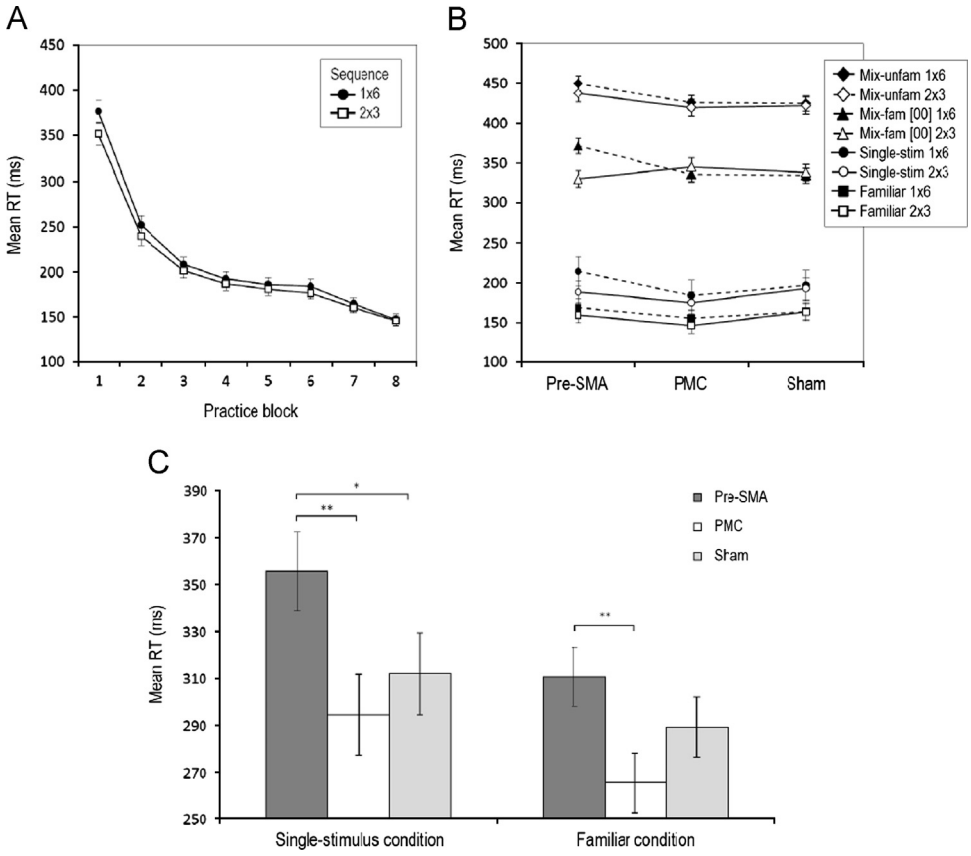

Fig. 3. Sequencing performance in the practice and test phase. *Panel A*: Mean RTs of the 1×6 and 2×3 sequences as a function of practice block. *Panel B*: Mean RTs of the 1×6 and 2×3 sequences as a function of test condition for the pre-SMA, PMC and sham groups. *Panel C*: Mean RTs for motor chunk initiation in the single-stimulus and familiar test conditions as a function of rTMS group (**$=p < .05$ and *$=p < .05$ when tested one-tailed). All error bars represent standard errors.

5.5 cm anterior to Cz—this distance closely resembles the location of the pre-SMA such as determined by Kennerley et al. (2004).

We used two control groups: one involving actual rTMS stimulation and one involving sham stimulation. Previous studies showed that when the non-dominant hand is used for performance – as is the case in the present study – the ipsilateral premotor cortex (PMC) is dominant for movement selection (e.g., Grafton, Hazeltine, and Ivry (2002), Gu et al. (2003), Schluter, Rushworth, Passingham, and Mills (1998)). Consequently, rTMS simulation of the PMC contralateral to the hand performing the movements should not affect sequencing performance and was therefore chosen as a first control site. The stimulation site for the right PMC was located 2 cm anterior and 1 cm medial to the motor hotspot (e.g., Bestmann, Baudewig, Siebner, Rothwell, and Frahm (2005), Bijsterbosch, Lee, Dyson-Sutton, Barker, and Woodruff (2011), Murase et al. (2005)). We included a second control group that involved sham stimulation, in which participants were treated as part of one of the two other groups, meaning that half of these participants experienced the (inactive) coil above the pre-SMA and the other half above the right PMC region.

The TMS coil was always positioned at the location of stimulation with the handle pointing backward and laterally at a 45 degree angle. Participants in the active rTMS groups were administered 20 min of 1 Hz rTMS stimulation at an intensity at 90% of each participant's individual MT. After the rTMS procedure, there was a 20-min rest period as the effects of off-line stimulation have been found most pronounced after such a pause (e.g., Verwey et al. (2002)).

2.5. Procedure

The experiment consisted of two sessions that took place on two consecutive days. Upon entering the lab on the first day of the experiment, participants filled out the screening questionnaire for TMS candidates (Rossi et al., 2009) and the Handedness Inventory (Annett, 1970) to determine whether they met the requirements for participation in the study. They then signed an informed consent form and received instructions regarding the task. Next, they performed the six practice blocks. Finally, participants completed an awareness questionnaire in which they were asked to write down their two sequences from memory and to recognize their sequences from a list of 12 alternatives. On the second day of the experiment, participants performed two additional practice blocks, after which the rTMS procedure was carried out. The participants then completed the four blocks of the test phase. The duration of the experiment was about 2 h per day for each participant.

3. Results

The rTMS procedure was well tolerated by all participants and no adverse events occurred during the experiment. We calculated mean response times (RTs) within the 2×3 and 1×6 sequences for every participant in each block of the practice and the test phase. RT was defined as the time between stimulus presentation and depression of the appropriate response key. Sequences in which an error was made – resulting in immediate abortion of the ongoing sequence – and the first two sequences of each (sub-) block were excluded from the RT analyses. RTs deviating more than 2.5 SDs from the mean RT of that sequence for each rTMS group in a particular (sub-)block were also excluded. This last procedure affected less than 1% of the data. For one participant in the sham group, mean RTs in the test phase deviated more than 3SD from the group mean and these were therefore not included in the analyses below.

3.1. Practice phase

A mixed ANOVA on RTs with Block (8) and Sequence (2; 2×3 vs. 1×6) as within-subject variables and rTMS group (3; pre-SMA vs. PMC vs. Sham) as a between-subject variable showed an effect of Block, $F(7, 308) = 310.12$, $p < .001$, $\eta_p^2 = .87$, indicating that sequencing performance improved with practice (cf. Fig. 3, panel A). The 2×3

sequence was performed faster than the 1 × 6 sequence (206 ms vs. 214 ms), $F(1, 44)=7.01$, $p<.05$, $\eta_p^2=.13$, but as Fig. 3 (panel A) illustrates this difference reduced as practice progressed, $F(7, 308)=4.65$, $p<.05$, $\eta_p^2=.10$. The absence of any significant effects of rTMS group indicates that the effects of rTMS reported below cannot be attributed to baseline differences between these groups ($ps>.44$).

We also analyzed participants' performance in terms of accuracy by means of a mixed ANOVA on proportions of correctly performed sequences with Practice block (8) and Sequence (2) as within-subject variables and rTMS group (3) as a between-subject variable. Results indicated that participants correctly executed more 1 × 6 sequences than 2 × 3 sequences (.90 vs. .88), $F(1, 44)=6.81$, $p<.05$, $\eta_p^2=.13$. This pattern was reversed in the first practice block compared to subsequent blocks, $F(7, 308)=2.49$, $p<.05$, $\eta_p^2=.05$. Across the practice phase, the mean proportion of correctly completed sequences across participants was never below.84. Like in the RT analysis, there were no main or interaction effects of rTMS group ($ps>.50$).

3.2. Test phase

3.2.1. Overall ANOVA

Results of a mixed ANOVA on RTs with Test condition (4; single-stimulus vs. familiar vs. mixed-familiar [00 sequences] vs. mixed-unfamiliar) and Sequence (2) as within-subject variables and rTMS group (3) as a between-subject variable showed that performance in the four test conditions differed substantially, $F(3, 129)=749.20$, $p<.001$, $\eta_p^2=.94$ (cf. Fig. 3, panel B). Across the rTMS groups, sequences were performed fastest in the single-stimulus and familiar conditions, which is in line with the notion of rapid performance in the chunking mode. However, sequences were performed slower in the single-stimulus condition than in the familiar condition (192 ms vs. 160 ms, respectively), $F(1, 43)=38.23$, $p<.001$, $\eta_p^2=.47$. This is in line with the idea that a race between response selection (i.e., direct S–R translations) and response triggering (i.e., on the basis of response-codes in the motor buffer) speeds up performance: As the selection of responses past the first was disabled in the single-stimulus condition, RTs slightly increased. Responses in the mixed-unfamiliar condition were slowest (430 ms), reflecting purely S–R based performance in the reaction mode. Planned comparisons further confirmed that sequences without deviants in the mixed-familiar condition were performed in the associative mode (343 ms), as they were performed faster than sequences in the mixed-unfamiliar condition, $F(1, 29)=273.35$, $p<.001$, $\eta_p^2=.86$, yet slower than sequences in the single-stimulus and familiar conditions, $Fs>246.29$, $ps<.001$, η_p^2s $>.85$. These findings are nicely in line with the core assumptions of the DPM (see Abrahamse et al. (2013)), as they support both the three-mode division and the race principle.

Results further showed that the 1 × 6 sequence was performed slower than the 2 × 3 sequence (286 ms vs. 277 ms), $F(1, 43)=11.49$, $p<.01$, $\eta_p^2=.21$. However, a Sequence x rTMS group interaction suggested that this difference between the 1 × 6 and 2 × 3 sequences varied for the three rTMS groups, $F(2, 43)=6.65$, $p<.05$, $\eta_p^2=.23$, while a Sequence × rTMS group × Test condition interaction suggested that this was further moderated by test condition, $F(6, 129)=2.56$, $p<.05$, $\eta_p^2=.10$ (Fig. 3, panel B).

To further investigate these interactions, we performed separate ANOVAs per rTMS group with Test condition (4) and Sequence (2) as within-subject variables. Results of the PMC and sham groups showed no main or interaction effects of sequence, $ps>.34$. However, results of the pre-SMA group revealed that the 1 × 6 sequence was performed slower than the 2 × 3 sequence (301 ms vs. 279 ms), $F(1, 15)=16.33$, $p<.01$, $\eta_p^2=.52$. In addition, there was a strong trend towards a Test condition x Sequence interaction, $F(3, 45)=3.02$, $p=.06$, $\eta_p^2=.16$, suggesting that the difference between the two sequences varied between test conditions. Detailed analyses were carried out to test whether more complicated sequences rely more on pre-SMA. These showed that the 1 × 6 sequence was performed slower than the 2 × 3 sequence in the single-stimulus, mixed-familiar and mixed-unfamiliar conditions, $ts>2.45$, $ps<.05$, but not in the familiar condition ($p=.16$; see Fig. 3, panel B). Overall, this indicates that the pre-SMA is more involved in the relatively complex 1 × 6 sequence than the 2 × 3 sequence, and this notion is elaborated upon below in the Discussion section. We now first continue with a number of more focused analyses that directly relate to the question whether the pre-SMA is involved in motor chunk initiation (cf. Kennerley et al., 2004).

3.2.2. Motor chunk initiation

As outlined in the introduction, we proposed that the pre-SMA is involved in the loading of motor chunks into the motor buffer when sequences are performed in the chunking mode (i.e., in the familiar and single-stimulus test conditions). To explore whether the initiation and execution of motor chunks differed amongst the rTMS groups, we first examined whether participants segmented their sequences into multiple motor chunks in the final practice block. We assumed that the first key press reflects initiation of the first motor chunk. Initiation of subsequent chunks is reflected in a key press within the sequence that is significantly slower than both its preceding and succeeding key presses (see Bo and Seidler (2009), Kennerley et al. (2004), Ruitenberg et al. (2012)). We ran one-tailed paired t-tests ($p<.05$) on RTs of the third, fourth and fifth key press of each sequence to evaluate whether the RT on a particular position in the sequence was significantly longer than the previous and subsequent RTs (the second and sixth key presses were not evaluated as such, because we assumed that they are always included in the first and last motor chunk, respectively). As chunking patterns are likely to differ for the two sequences that a participant performed, we analyzed the 1 × 6 and 2 × 3 sequences separately.

This procedure revealed that 27 (of the 47) participants segmented their 1 × 6 sequence and 39 participants segmented their 2 × 3 sequence into multiple motor chunks. RTs of key presses that were classified as being the first key press of a motor chunk (i.e., the first key press and chunk points) were averaged to compute the mean initiation RT per participant per sequence. The RTs of the remaining key presses were averaged to compute the mean execution RT (reflecting mostly execution processes). We subjected these RTs to a mixed ANOVA with Test condition (2; single-stimulus vs. familiar[2]), Sequence (2) and Phase (2; chunk initiation vs. execution of other key presses) as within-subject variables and rTMS group (3) as a between-subject variable. In addition to the effects of Test condition and Sequence found in the above analyses, results showed that – as expected – initiating motor chunks took longer than executing other key presses within the chunks (305 ms vs. 113 ms), $F(1, 43)=568.12$, $p<.001$, $\eta_p^2=.93$.

A Phase × rTMS group interaction indicated a differential involvement of the pre-SMA in initiation and execution, $F(2, 43)=3.57$, $p<.05$, $\eta_p^2=.14$. Planned comparisons revealed that initiation differed between rTMS groups, $F(2, 43)=3.42$, $p<.05$, $\eta_p^2=.13$, while execution did not ($p=.40$). Most crucially, results from the ANOVA showed a Test condition × Phase × rTMS group interaction, $F(2, 43)=3.94$, $p<.05$, $\eta_p^2=.15$, indicating that the differences between rTMS groups on initiation varied between the test conditions. Further planned

[2] Motor chunking was assumed to only be potentially witnessed in these conditions.

comparisons revealed that motor chunk initiation in the single-stimulus test condition was different for the various rTMS groups, $F(2, 43)=3.43$, $p<.05$, $\eta_p^2=.14$ (Fig. 3, panel C). As expected on the basis of Kennerley et al. (2004), motor chunk initiation was slowed by rTMS stimulation of the pre-SMA compared to both the sham group, $F(1, 29)=3.02$, $p<.05$, $\eta_p^2=.09$ (one-tailed), and the PMC group, $F(1, 29)=5.22$, $p<.05$, $\eta_p^2=.15$. Motor chunk initiation was not affected by rTMS stimulation of the PMC compared to the sham group ($p=.39$). With regard to the familiar condition, there was a strong tendency for initiation to be affected by the rTMS group, $F(2, 45)=3.09$, $p=.058$, $\eta_p^2=.12$. Again, initiation in the pre-SMA group was slower than initiation in the PMC group, $F(1, 30)=5.35$, $p<.05$, $\eta_p^2=.15$. In contrast to the single-stimulus condition, however, we did not observe a significant difference between the pre-SMA and the sham groups for the familiar condition ($p=.25$), although initiation times were indeed numerically higher in the pre-SMA. Motor chunk initiation in the PMC and sham groups did not differ ($p=.18$).

A final ANOVA on RTs with Test condition (2), Chunk point (2; first key press vs. chunk within the sequence), Sequence (2) and rTMS group (3) showed no significant interactions between Chunk point and rTMS group ($ps>.26$), indicating that both initiation of a motor chunk at the start of the sequence and of a motor chunk half-way through the sequence (cf. Kennerley et al., 2004) were similarly affected by rTMS stimulation of the pre-SMA. Overall, then, the results suggest that the initiation of motor chunks – but not the execution of elements within motor chunks – is impaired when rTMS is applied to the pre-SMA.

3.2.3. Stimulus–response translation, priming, and racing

In this section we explore the involvement of the pre-SMA in direct S–R translation in the reaction mode, association-based priming in the associative mode, and racing between two response-generation processes in the chunking mode. First, to analyze the contribution of the pre-SMA to direct S–R translations in the reaction mode, we performed a mixed ANOVA on RTs in the mixed-unfamiliar test condition with Sequence (2) as a within-subject variable and rTMS group (3) as a between-subject variable. Results showed that the 1×6 sequence was performed slower than the 2×3 sequence, $F(1, 44)=7.43$, $p<.01$, $\eta_p^2=.14$, but there were no effects of rTMS group ($ps>.23$). This suggests that the pre-SMA is not involved in selecting individual responses on the basis of S–R translations by the cognitive processor.

Second, to explore pre-SMA involvement in the associative mode, we analyzed the performance of the sequences without deviants in the mixed-familiar condition. We did not include the first key press of the sequences in these analyses, as this key press cannot be facilitated through priming by a previous stimulus–response event (i.e., associative mechanism). Results of a mixed ANOVA on RTs in the mixed-familiar (00 sequences) test condition with Sequence (2) as a within-subject variable and rTMS group (3) as a between-subject variable showed an interaction between Sequence and rTMS group, $F(2, 44)=4.72$, $p<.05$, $\eta_p^2=.18$. Like in the previous section, detailed analysis showed that execution of the 1×6 sequence was generally slower than that of the 2×3 sequence for participants in the pre-SMA group (354 ms vs. 312 ms), $F(1, 15)=11.35$, $p<.01$, $\eta_p^2=.43$. However, there were no performance differences between the sequences in the other rTMS groups (323 ms vs. 328 ms for the PMC group; 318 ms vs. 321 ms for the sham group; $ps>.70$). As there was no main effect of rTMS ($p=.54$), we found no indication for pre-SMA involvement in the associative mode[3].

[3] One could argue that for the associative mode, the effects of rTMS group should be explored for the performance difference between [00] sequences in the mixed-familiar and either the unfamiliar or familiar blocks. No main effects of rTMS group were observed for those analyses either ($ps>.59$).

Table 1
Explicit sequence knowledge. The numbers and the corresponding percentages of participants per rTMS group who correctly wrote down their 1×6 and 2×3 sequences immediately following the practice phase on the first day of the experiment ('recall' columns), and recognized their sequences from a set of 12 alternatives ('recognition' columns).

	Recall		Recognition	
	1×6	2×3	1×6	2×3
Pre-SMA	13 (81%)	13 (81%)	15 (94%)	15 (94%)
PMC	10 (63%)	12 (75%)	15 (94%)	15 (94%)
Sham	11 (73%)	14 (93%)	15 (100%)	15 (100%)

Third, to test the involvement of the pre-SMA in racing in the chunking mode, we examined the performance difference between sequences in the single-stimulus and familiar test conditions. Again, the first key press was excluded from the analysis. We performed a mixed ANOVA on RT differences between the single-stimulus and familiar test conditions with Sequence (2) as a within-subject variable and rTMS group (3) as a between-subject variable. Results showed no significant main or interaction effects ($ps>.24$), thus revealing no indications for pre-SMA involvement in the race between two response generation processes in the familiar condition[4]. Overall, these analyses indicate no pre-SMA involvement in either direct S–R translation (reaction mode, associative mode, racing) or association-based priming (associative mode).

3.2.4. Accuracy

The mean proportion of correctly performed sequences was 0.84 in the single-stimulus condition, 0.86 in the familiar condition, 0.81 in the mixed-familiar condition and 0.84 in the mixed-unfamiliar condition. Results of a mixed ANOVA on these proportions with Test condition (4) and Sequence (2) as within-subject variables and rTMS group (3) as a between-subject variable showed there was a tendency for accuracy to differ between the test conditions, $F(3, 132)=2.62$, $p=.08$, $\eta_p^2=.05$. This indicated that accuracy was highest in the familiar condition (removing this condition from the analysis resulted in the absence of the effect, $p=.34$). There were no other main or interaction effects ($ps>.14$).

3.3. Explicit sequence knowledge

Analyses of the awareness questionnaire showed no differences in recall or recognition of the 1×6 and 2×3 sequences between the rTMS groups (χ^2s$(2)<1.4$, $ps>.38$; see Table 1). This indicates that the observed performance differences cannot be attributed to group differences with regard to explicit sequence knowledge.

4. Discussion

In the present study we tested and confirmed the core assumptions of the DPM in a single experimental design. Most critically, we confirmed that discrete movement sequences can be performed in three execution modes (cf. Verwey & Abrahamse, 2012), and that key-specific stimuli continue to support execution of well-practiced, familiar movement sequences (cf. Verwey et al., 2010, 2014). From there, we set out to explore the involvement of

[4] Removing chunk points from the analysis did not yield a different pattern of results ($ps>.15$).

the pre-SMA in the various functions attributed to DPM's cognitive processor (Abrahamse et al., 2013; Verwey, 2001). These functions include selecting individual responses in the reaction mode and the associative mode, and initiating motor chunks in the chunking mode. We applied 20 min 1 Hz off-line rTMS to the pre-SMA and compared participants' sequencing performance in the test phase with that of participants in two control groups in which either the PMC was stimulated or sham stimulation was used.

In a nutshell, we observed (I) that rTMS stimulation of the pre-SMA disrupts motor chunk initiation in the chunking mode. Interestingly, this disruption was observed both at the start of each sequence and at the initiation of chunks halfway through the sequence. This replicates in a single experiment the findings of Experiment 2 (disruption of motor chunk initiation halfway through the sequence) and Experiments 3 and 4 (disruption of motor chunk initiation at the start of the sequence) of the study by Kennerley et al. (2004). Moreover, it extends this study to conditions with substantially more practice and shorter sequences. As expected, execution of the elements within a motor chunk was not affected.

Additionally, we observed (II) that rTMS stimulation of the pre-SMA especially affects performance of 1×6 sequences compared to the 2×3 sequences, suggesting a role in managing sequence complexity in line with previous fMRI work (Boecker et al., 1998; Gerloff et al., 1997; Grafton et al., 1998). No indications were found for pre-SMA involvement in either direct S–R translation in the (III) reaction and (IV) associative modes, or (V) in the race between the cognitive processor and the motor system. Below, we will elaborate on these findings.

4.1. The neural substrate of the cognitive processor

Even though the DPM's cognitive processor refers to a set of (non-motoric) processes, the label was never intended to suggest that all these processes can ultimately be pinned down onto one particular network. Indeed, the current results suggest that some processes are related to a network that involves the pre-SMA (i.e., motor chunk initiation, managing sequence complexity), while other processes are not (i.e., online S–R translation, priming of responses). This is in line with the notion that a large number of brain areas are found to be involved in sequence skill studies, and in different combinations.

With respect to the observation that the pre-SMA differentially affected the 1×6 and 2×3 sequences, it should be noted that this aligns well with previous findings that activation in this area is greater for complex than for relatively simple sequences (Boecker et al., 1998; Gerloff et al., 1997; Grafton et al., 1998). We here propose that the pre-SMA is involved in managing sequence complexity – or even more generally in task difficulty – and that the crucial difference between the 1×6 and the 2×3 sequences is the need for stronger initial (pre-SMA driven) preparation in the 1×6 sequence. Hence, for the 2×3 sequence one can suffice with preparing three elements (which are then executed twice), while for the 1×6 sequence up to six elements need to be prepared independently. The slowed 1×6 sequence can be explained by less successful – or incomplete – preparation processes with pre-SMA disruption. The observation that this modulation by the pre-SMA was not present for the familiar condition is not easy to account for, though. One may argue that this could be tentatively explained by assuming that the preparation process was more or less the same with extensive practice for both sequences, because they both involved the retrieval from long-term memory of a single motor chunk—but then why was the effect present in the single-stimulus condition? Maybe the single-stimulus condition triggered more controlled preparation because participants were aware that stimuli were not present to assist? This study only allows speculating about such, and we believe that further research is required to understand the precise relationship between pre-SMA activity and sequence complexity.

The pre-SMA has already been claimed to be increasingly involved in the production of sequential action as the sequence becomes more familiar (e.g., Halsband and Lange (2006), Kennerley et al. (2004), Wymbs and Grafton (2013)). This notion fits well with the here observed link between the pre-SMA and preparation/initiation processes of motor chunks—because for this, one needs sufficient practice to develop motor chunks in the first place. For this interpretation to be successfully applied to DSP performance, we believe it was important to show here, in direct extension of the findings of Kennerley et al. (2004), that both the initiation of the first motor chunk of a sequence (i.e., sequence initiation) and initiation of subsequent motor chunks within one sequence were slowed by rTMS stimulation of the pre-SMA.

The pre-SMA may operate at a hierarchically higher level than the SMA proper. That is, the pre-SMA may be involved in the loading of motor chunks into the motor buffer while the SMA proper is more directly involved in the execution of individual sequence elements. The latter is in line with the finding of Verwey et al. (2002), that rTMS stimulation of the SMA slowed all elements of a discrete keying sequence. Such a divide in functionality fits well with anatomical findings that the SMA is densely connected to motor areas, while the pre-SMA is connected to frontal areas (e.g., Picard and Strick, (2001)). We tentatively suggest, then, that at advanced skill levels the pre-SMA activates the chunk-specific long-term memory representations (i.e., load and initiate the motor buffer), after which execution may be controlled by more posterior motor regions such as the SMA and the primary motor cortex (M1; e.g., Abrahamse et al. (2013), Karni et al. (1998), Kennerley et al. (2004), Ungerleider, Doyon, and Karni (2002)).

One could argue that the difference between our study and the one by Verwey et al. (2002) is not related to differential roles of the pre-SMA and SMA in discrete sequence skill, but rather to the fact that in the latter only the first stimulus of a sequence was presented (whereas in the current study all stimuli were presented across the training and most of the test conditions). However, in the present study we also included a single-stimulus condition and still observed effects of rTMS only on initiation and not execution. This finding rules out a fundamental role of differences in stimulus presentation.

Alternatively, one could argue that the number of practice trials accounted for the discrepancy in findings between the Verwey et al. (2002) and the current study (involving 210 vs. 720 repetitions per sequence, respectively), and thus that the difference between pre-SMA and SMA is (merely) related to the development and use of motor chunks. In principle this is a valid argument. However, studies have shown that even with moderate amounts of practice (i.e., about 150 repetitions per sequence) people perform their sequences based on motor chunks—as witnessed among others by a clear chunking pattern and a functional dissociation between initiation and execution key-presses with various manipulations (e.g., De Kleine and Verwey (2009a, 2009b), Ruitenberg et al. (2013)). We thus believe that pre-SMA and SMA relate to different roles in discrete sequence skill, but future research should directly test this hypothesis.

Overall, our study suggests that processes related to motor chunk initiation – both at the start of and within an ongoing sequence – and sequence complexity are (partly) mapped on a shared neural substrate that involves the pre-SMA. These results nicely replicate and extend earlier work that showed the involvement of pre-SMA in motor chunk initiation (Kennerley et al., 2004) or in dealing with sequence complexity (Boecker et al., 1998; Gerloff et al., 1997; Grafton et al., 1998). It must be noted, though,

that our arguments for the alleged role of the pre-SMA may be taken with some caution because we did not use (anatomical or functional) MRI to confirm the localization of the stimulation site. Moreover, we do not know whether current findings relate to a local disruption of the pre/SMA or rather to disruption of a distributed neuronal network that included the pre-SMA. A final limitation concerns the spatial resolution of the rTMS pulses: It is possible that areas that are anatomically closely connected to the pre-SMA were affected by the pulses as well. However, despite these limitations, the fact that we were able to replicate findings from earlier studies (Boecker et al., 1998; Gerloff et al., 1997; Grafton et al., 1998; Kennerley et al., 2004) at least strongly suggests that we correctly localized and targeted the pre-SMA.

4.2. Additional findings

Even though the focus of the current study was on the neural substrate of the cognitive processor, we here would like to emphasize that the current study – within a single design – replicated a number of findings from earlier DSP studies that lay at the core of the DPM. First, in line with Verwey and Abrahamse (2012) we show that sequences can be executed in three different modes: the reaction mode, the associative mode, and the chunking mode. Second, we provide support for the notion that a cognitive processor and a motor processor are both racing to produce the next response while executing familiar discrete movement sequences (cf. Verwey, 2001; Verwey et al., 2010, 2014). Third, we provide further evidence that the initiation of a motor chunk can be empirically dissociated from mere execution of responses within the motor chunk (cf. De Kleine & Verwey, 2009a, 2009b), thus supporting the idea that it reflects other (or additional) processes—most likely preparatory in nature. As such the current study provides strong support for (the most crucial assumptions of) the DPM.

The absence in the current study of any effects on sequencing performance after rTMS of the right PMC corroborates findings from the MRI study by Grafton et al. (2002) that contralateral PMC is hardly involved when the non-dominant left hand is used for sequence performance, and thus supports that this area is a reliable control site for future rTMS studies on sequence skill. It also suggests that the left PMC is involved in sequential action irrespective of the hand used. As a next step, it would be interesting to use the right PMC as a control site for zooming in on the role of the left PMC. In our recent review (Abrahamse et al., 2013), we speculated that loops between the basal ganglia and PMC may be involved in direct stimulus–response translation during sequence skill, which would be in line with findings from a number of human and monkey studies that indicated PMC involvement (Grafton et al., 2002; Halsband, Matsuzaka, & Tanji, 1994; Mushiake, Inase, & Tanji, 1991; Schluter et al., 1998). Future studies should further address the issues of hand- and hemisphere-dominance to increase our understanding of the PMC in sequential action, and should clarify which neural substrates underlie sequencing performance in the reaction and associative modes. For example, the left-PMC could be involved in the S–R translation process that takes place during sequencing performance in the reaction and associative modes—and possibly the racing on basis of S–R translations in the chunking mode.

4.3. Conclusions

The current study provides a few pieces of the unsolved puzzle of the neural substrate underlying (discrete) sequence skill. Specifically, we zoomed in on the pre-SMA and explored its contribution to a number of functions that we theorized to be at play during sequencing performance. Most importantly, we demonstrate that the pre-SMA is involved in the selection and initiation of motor chunks, and in dealing with the cognitive demands of sequence complexity. Here we have framed these results within the so-called dual processor model (Abrahamse et al., 2013), but we believe they are of great relevance for sequential motor skill in general. Future studies should zoom in on the involvement of other brain areas (such as ipsilateral PMC in non-dominant hand use) across the various functions outlined by DPM.

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References

Abrahamse, E. L., Jimenez, L., Verwey, W. B., & Clegg, B. (2010). Representing serial action and perception. *Psychonomic Bulletin & Review*, *17*, 603–623.

Abrahamse, E. L., Ruitenberg, M. F. L., De Kleine, E., & Verwey, W. B. (2013). Control of automated behaviour: Insights from the discrete sequence production task. *Frontiers in Human Neuroscience*, *7*, 82, http://dx.doi.org/10.3389/fnhum.2013.00082.

Annett, M. (1970). A classification of hand preference by association analysis. *British Journal of Psychology*, *61*, 303–321.

Ashby, F. G., Turner, B. O., & Horvitz, J. C. (2010). Cortical and basal ganglia contributions to habit learning and automaticity. *Trends in Cognitive Science*, *14*, 208–215.

Bestmann, S., Baudewig, J., Siebner, H. R., Rothwell, J. C., & Frahm, J. (2005). BOLD MRI responses to repetitive TMS over human dorsal premotor cortex. *NeuroImage*, *28*, 22–29.

Bijsterbosch, J. D., Lee, K.-H., Dyson-Sutton, W., Barker, A. T., & Woodruff, P. W. R. (2011). Continuous theta burst stimulation over the left pre-motor cortex affects sensorimotor timing accuracy and supraliminal error correction. *Brain Research*, *1410*, 101–111.

Bo, J., & Seidler, R. D. (2009). Visuospatial working memory capacity predicts the organization of acquired explicit motor sequences. *Journal of Neurophysiology*, *101*, 3116–3125.

Boecker, H., Dagher, A., Ceballos-Baumann, O., Passingham, R. E., Samuel, M., Friston, K. J., et al. (1998). Role of the human rostral supplementary motor area and the basal ganglia in motor sequence control: Investigations with $H_2{}^{15}O$ PET. *Journal of Neurophysiology*, *79*, 1070–1080.

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E. M., Hallett, M., et al. (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, *48*, 1398–1403.

De Kleine, E., & Verwey, W. B. (2009a). Representations underlying skill in the discrete sequence production task: Effect of hand used and hand position. *Psychological Research*, *73*, 685–694.

De Kleine, E., & Verwey, W. B. (2009b). Motor learning and chunking in dyslexia. *Journal of Motor Behavior*, *41*, 331–337.

Gerloff, C., Corwell, B., Chen, R., Hallett, M., & Chen, L. G. (1997). Stimulation over the human supplementary motor area interferes with the organization of future elements in complex motor sequences. *Brain*, *120*, 1587–1602.

Grafton, S. T., Hazeltine, E., & Ivry, R. B. (1998). Abstract and effector-specific representations of motor sequences identified with PET. *The Journal of Neuroscience*, *18*, 9420–9428.

Grafton, S. T., Hazeltine, E., & Ivry, R. B. (2002). Motor sequence learning with the nondominant left hand. A PET functional imaging study. *Experimental Brain Research*, *146*, 369–378.

Gu, Y., Zang, Y., Weng, X., Jia, F., Li, E., & Wang, J. (2003). Involvement of secondary motor areas in externally-triggered single-finger movements of dominant and non-dominant hands. *Chinese Science Bulletin*, *48*, 1569–1575.

Halsband, U., & Lange, R. K. (2006). Motor learning in man: A review of functional and clinical studies. *Journal of Physiology (Paris)*, *99*, 414–424.

Halsband, U., Matsuzaka, Y., & Tanji, J. (1994). Neuronal activity in the primate supplementary, pre-supplementary and premotor cortex during externally and internally instructed sequential movements. *Neuroscience Research*, *20*, 149–155.

Hikosaka, O., Nakahara, H., Rand, M. K., Sakai, K., Lu, X., Nakamura, K., et al. (1999). Parallel neural networks for learning sequential procedures. *Trends in Neuroscience*, *22*, 464–471.

He, S. Q., Dum, R. P., & Strick, P. L. (1995). Topographic organization of corticospinalprojections from the frontal lobe: Motor areas on the medial surface of the hemisphere. *Journal of Neuroscience*, *15*, 3284–3306.
Jenkins, I. H., Brooks, D. J., Nixon, P. D., Frackowiak, R. S., & Passingham, R. E. (1994). Motor sequence learning: A study with positron emission tomography. *Journal of Neuroscience*, *14*, 3775–3790.
Jenkins, I. H., Jahanshahi, M., Jueptner, M., Passingham, R. E., & Brooks, D. J. (2000). Self-initiated versus externally triggered movements. II. The effect of movement predictability on regional cerebral blood flow. *Brain*, *123*, 1216–1228.
Karni, A., Meyer, G., Rey-Hipolito, C., Jezzard, P., Adams, M. M., Turner, R., et al. (1998). The acquisition of skilled motor performance: Fast and slow experience-driven changes in primary motor cortex. *Proceedings of the National Academy of Science USA*, *95*, 861–868.
Klapp, S. T. (1995). Motor response programming during simple and choice-reaction time: The role of practice. *Journal of Experimental Psychology: Human Perception & Performance*, *21*, 1015–1027.
Klapp, S. T. (2003). Reaction time analysis of two types of motor preparation for speech articulation: Action as a sequence of chunks. *Journal of Motor Behaviour*, *35*, 135–150.
Keele, S. W., Ivry, R., Mayr, U., Hazeltine, E., & Heuer, H. (2003). The cognitive and neural architecture of sequence representation. *Psychological Review*, *110*, 316–339.
Kennerley, S. W., Sakai, K., & Rushworth, M. F. S. (2004). Organization of action sequences and the role of the pre-SMA. *Journal of Neurophysiology*, *91*, 978–993.
Luppino, G., Matelli, M., Camarda, R., & Rizzolatti, G. (1993). Corticocortical connections of area F3 (SMA-proper) and area F6 (pre-SMA) in the macaque monkey. *Journal of Comparative Neurology*, *338*, 114–140.
Mantovani, A., Simpson, H. B., Fallon, B. A., Rossi, S., & Lisanby, S. H. (2010). Randomized sham-controlled trial of repetitive transcranial magnetic stimulation in treatment-resistant obsessive-compulsive disorder. *The International Journal of Neuropsychopharmacology*, *13*, 217–227.
Murase, N., Rothwell, J. C., Kaji, R., Urushihara, R., Nakamura, K., Murayama, K., et al. (2005). Subthreshold low-frequency repetitive transcranial magnetic stimulation over the premotor cortex modulates writer's cramp. *Brain*, *128*, 104–115.
Mushiake, H., Inase, M., & Tanji, J. (1991). Neuronal activity in the primate premotor, supplementary, and precentral motor cortex during visually guided and internally determined sequential movements. *Journal of Neurophysiology*, *66*, 705–718.
Penhune, V. B., & Steele, C. J. (2012). Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. *Behavioral Brain Research*, *226*, 579–591.
Picard, N., & Strick, P. L. (1996). Medial wall motor areas: A review of their location and functional activation. *Cerebral Cortex*, *6*, 342–353.
Picard, N., & Strick, P. L. (2001). Imaging the premotor areas. *Current Opinion in Neurobiology*, *11*, 663–672.
Rosenbaum, D. A., Kenny, S. B., & Derr, M. A. (1983). Hierarchical control of rapid movement sequences. *Journal of Experimental Psychology: Human Perception & Performance*, *9*, 86–102.
Rossi, S., Hallet, M., Rossini, P. M., & Pascual-Leone, A. (2009). the safety of TMS consensus group.. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, *120*, 2008–2039.
Rossi, S., & Rossini, P. M. (2004). TMS in cognitive plasticity and the potential for rehabilitation. *TRENDS in Cognitive Sciences*, *8*, 273–279.
Ruitenberg, M. F. L., Abrahamse, E. L., De Kleine, E., & Verwey, W. B. (2012). Context-dependent motor skill: Perceptual processing in memory-based sequence production. *Experimental Brain Research*, *222*, 31–40.
Ruitenberg, M. F. L., Abrahamse, E. L., & Verwey, W. B. (2013). Sequential motor skill in preadolescent children: The development of automaticity. *Journal of Experimental Child Psychology*, *115*, 607–623.
Ruitenberg, M. F. L., De Kleine, E., Van der Lubbe, R. H. J., Verwey, W. B., & Abrahamse, E. L. (2012). Context-dependent motor skill and the role of practice. *Psychological Research*, *76*, 812–820.
Sakai, K., Kitaguchi, K., & Hikosaka, O. (2003). Chunking during human visuomotor sequence learning. *Experimental Brain Research*, *152*, 229–242.
Schluter, N. D., Rushworth, M. F. S., Passingham, R. E., & Mills, K. R. (1998). Temporary interference in human lateral premotor cortex suggests dominance for the selection of movements. A study using transcranial magnetic stimulation. *Brain*, *121*, 785–799.
Schutter, D. J. L. G., & van Honk, J. (2006). A standardized motor threshold estimation procedure for transcranial magnetic stimulation research. *Journal of ECT*, *22*, 176–178.
Toni, I., Krams, M., Turner, R., & Passingham, R. E. (1998). The time course of changes during motor sequence learning: A whole-brain fMRI study. *NeuroImage*, *8*, 50–61.
Ungerleider, L. G., Doyon, J., & Karni, A. (2002). Imaging brain plasticity during motor skill learning. *Neurobiology of Learning and Memory*, *78*, 553–564.
Verwey, W. B. (1999). Evidence for a multistage model of practice in a sequential movement task. *Journal of Experimental Psychology: Human Perception and Performance*, *25*, 1693–1708.
Verwey, W. B. (2001). Concatenating familiar movement sequences: The versatile cognitive processor. *Acta Psychologica*, *106*, 69–95.
Verwey, W. B. (2003). Processing modes and parallel processors in producing familiar keying sequences. *Psychological Research*, *67*, 106–122.
Verwey, W. B., & Abrahamse, E. L. (2012). Distinct modes of executing movement sequences: Reacting, associating, and chunking. *Acta Psychologica*, *140*, 274–282.
Verwey, W. B., Abrahamse, E. L., & De Kleine, E. (2010). Cognitive processing in new and practiced discrete keying sequences. *Frontiers in Psychology*, *1*, 1–13.
Verwey, W. B., Abrahamse, E. L., DeKleine, E., & Ruitenberg, M. F. L. (2014). Evidence for graded central processing resources in a sequential movement task. *Psychological Research*, *78*, 70–83.
Verwey, W. B., Abrahamse, E. L., & Jiménez, L. (2009). Segmentation of short keying sequences does not spontaneously transfer to other sequences. *Human Movement Science*, *28*, 348–361.
Verwey, W. B., & Eikelboom, T. (2003). Evidence for lasting sequence segmentation in the discrete sequence-production task. *Journal of Motor Behavior*, *35*, 171–181.
Verwey, W. B., Lammens, R., & Van Honk, J. (2002). On the role of the SMA in the discrete sequence production task: A TMS study. *Neuropsychologia*, *40*, 1268–1276.
Wang, Y., Isoda, M., Matsuzaka, Y., Shima, K., & Tanji, J. (2005). Prefrontal cortical cells projecting to the supplementary eye field and presupplementary motor area in the monkey. *Neuroscience Research*, *53*, 1–7.
Wassermann, E. M., Grafman, J., Berry, C., Hollnagel, C., Wild, K., Clark, K., et al. (1996). Use and safety of a new repetitive transcranial magnetic stimulator. *Electroencephalography and Clinical Neurophysiology*, *101*, 412–417.
Wymbs, N. F., Bassett, D. S., Mucha, P. J., Porter, M. A., & Grafton, S. T. (2012). Differential recruitment of the sensorimotor putamen and frontoparietal cortex during motor chunking in humans. *Neuron*, *74*, 936–946.
Wymbs, N. F., & Grafton, S. T. (2013). Contributions from the left PMd and the SMA during sequence retrieval as determined by depth of training. *Experimental Brain Research*, *224*, 49–58.