Organization of Action Sequences and the Role of the Pre-SMA

Steve W. Kennerley,1,2 K. Sakai,3 and M.F.S. Rushworth1,2
1Department of Experimental Psychology, University of Oxford, Oxford OX1 3UD; and 2Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), Department of Neurology, University of Oxford, Oxford OX3 9DU; and 3Wellcome Department of Cognitive Neurology, Institute of Neurology, London, WC1N 3BG, United Kingdom

Submitted 8 July 2003; accepted in final form 20 October 2003

Kennerley, Steve W., K. Sakai, and M.F.S. Rushworth. Organization of action sequences and the role of the pre-SMA. J Neurophysiol 91: 978–993, 2004. First published October 22, 2003; 10.1152/jn.00651.2003. To understand the contribution of the human presupplementary motor area (pre-SMA) in sequential motor behavior, we performed a series of finger key-press experiments. Experiment 1 revealed that each subject had a spontaneous tendency to organize or “chunk” a long sequence into shorter components. We hypothesized that the pre-SMA might have a special role in initiating each chunk but not at other points during the sequence. Experiment 2 therefore examined the effect of 0.5-s, 10-Hz repetitive transcranial magnetic stimulation (rTMS) directed over the pre-SMA. As hypothesized, performance was disrupted when rTMS was delivered over the pre-SMA at the beginning of the second chunk but not when it was delivered in the middle of a chunk. Contrary to the hypothesis, TMS did not disrupt sequence initiation. Experiments 3 and 4 examined whether the very first movement of a sequence could be disrupted under any circumstances. Pre-SMA TMS did disrupt the initiation of sequences but only when subjects had to switch between sequences and when the first movement of each sequence was not covertly instructed by a learned visuomotor association. In conjunction, the results suggest that for overlearned sequences the pre-SMA is primarily concerned with the initiation of a sequence or sequence chunk and the role of the pre-SMA in sequence initiation is only discerned when subjects must retrieve the sequence from memory as a superordinate set of movements without the aid of a visuomotor association. Control experiments revealed such effects were not present when rTMS was applied over the left dorsal premotor cortex.

INTRODUCTION

A fundamental aspect of human motor behavior is our ability to organize actions in a specific spatiotemporal order to achieve goal-directed behavior. The medial frontal cortex has been implicated in such sequencing of actions. Recent studies have particularly emphasized the sequencing role of the presupplementary motor area (pre-SMA), located in the rostromedial aspect of Brodmann’s area 6, within the medial frontal region. Regional cerebral blood flow and blood oxygenation-level-dependent (BOLD) signals in the pre-SMA change when sequences are learned (Hazeltine et al. 1997; Hikosaka et al. 1996; Jenkins et al. 1994; Sakai et al. 1998, 1999) and lesions, pharmacological inactivation, or transcranial magnetic stimulation (TMS) in this area disrupt the performance of well-learned sequences (Chen et al. 1995; Gerloff et al. 1997; Halsband 1987; Muri et al. 1994, 1995; Shima and Tanji 1998a).

Pre-SMA cells modulate their activity when sequences are performed, but in many studies, such modulation is transient and may only occur at certain points within a well-learned sequence (Nakamura et al. 1998; Shima and Tanji 2000; Shima et al. 1996). Nakamura et al. (1998) taught their monkeys ten movement sequences by teaching them a “hyperset” constructed from five pairs or “sets” of movements. These authors found extensive pre-SMA activity during the learning of a sequence. However, once a sequence was well learned, the activity of pre-SMA cells was greatly reduced, but any remaining pre-SMA activity was often confined to just the very first trial (or even the 1st set) of a well-learned hyperset. This suggests pre-SMA neurons are particularly interested in initiating sequences or component parts of sequences. Shima et al. (1996) similarly reported that many (25%) neurons in the pre-SMA fire in relation to just the first movement of a sequence. Moreover the firing pattern seemed to indicate a role in updating the sequence because the first movement-related activity was most prominent when the sequence changed and a new sequence had to be performed. Matsuzaka et al. (1996) also reported that many (31%) pre-SMA neurons fire when monkeys have to change and update a movement plan. Inactivation of the pre-SMA (Shima and Tanji 1998a) has demonstrated an essential role of the pre-SMA when a sequence is first retrieved from memory.

The more posterior SMA proper, together with the pre-SMA, may code for intervals between specific movements within a sequence and the rank order of sequence movements. Rank order and interval selective neurons are more prevalent in the pre-SMA and SMA, respectively, but both types of neuron are found in both areas (Shima and Tanji 2000). The pre-SMA, however, may be unique in making certain intermittent contributions to action sequences; it may be required when the sequence is first initialized, but subsequently it may only be of most importance at further points of higher level re-organization or re-direction of the sequence but not on the execution of every movement.

Reaction time (RT) analyses suggest that sequences exhibit two aspects of organizational structure. First, higher-level organizational processes are apparent when sequences are initiated. Before subjects make the first movement in a sequence, they are preparing aspects of the entire sequence of movements not just the first single movement. Sternberg et al. (1978) demonstrated that the RT of the first movement of a sequence increased as the total sequence length increased. It is possible that the prominent pre-SMA activity prior to the initiation of a sequence (Nakamura et al. 1998; Shima and Tanji 2000; Shima et al. 1996) is related to the process of sequence organization and initiation studied by Sternberg et al. (1978) rather than just the selection or execution of the first movement of the sequence.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: S. W. Kennerley, Dept. of Experimental Psychology, University of Oxford, South Parks Road, Oxford, OX1 3UD UK (E-mail: steve.kennerley@psy.ox.ac.uk).
A second aspect of sequence organization is sometimes apparent after the sequence is initiated. Several behavioral studies on sequence learning have suggested movement sequences are performed as if they consisted of subsequences or “chunks” (Koch and Hoffmann 2000; Nissen and Bullemer 1987; Perruchet and Amorim 1992; Povel and Collard 1982; Restle and Burnside 1972; Rosenbaum et al. 1983; Verwey 2001). The start of a chunk is indicated by a movement with a longer reaction time. In general, such investigations have imposed statistical or relational patterns (Koch and Hoffmann 2000) between the elements of a sequence such as repetitions of elements within the sequence, grouping elements of the sequence together by imposing pauses between certain elements, or transposing certain elements within a sequence. These imposed structure patterns have the effect of creating artificial “chunk points.” Thus it is not clear whether humans will spontaneously organize a random sequence into groups or chunks in the absence of such statistical or relational structure patterns. Such spontaneous organizational patterns in random sequences have proven difficult to discern at the group level because of highly individual organizational strategies. Verwey et al. (2003) demonstrated that at the group level, organizational patterns of sequences become more variable as the sequences become less structured. However, if such organizational strategies emerge as a genuine aspect of visuomotor sequence learning, possibly to overcome limitations of working memory, then it is possible that this process may also be under the control of the pre-SMA given its reciprocal connections with the prefrontal cortex (Bates and Goldman-Rakic 1993; Lu et al. 1994; Luppino et al. 1993).

Given the limitations of working memory (Miller 1956), experiment 1 sought evidence for the spontaneous organization of long sequences of finger key-press movements into component units or chunks. We taught subjects a bimanual sequence of 12 alternating movements until they could perform the sequence from memory (Fig. 1). Such a long bimanual sequence was chosen for two reasons: a pilot study demonstrated that an eight-movement sequence does not necessitate organization of a sequence into subsequences and bimanual sequences (as opposed to unimanual sequences) increase the number of possible movements in a sequence, thus reducing statistical or relational structure patterns that could influence chunking. A consistent pattern of increased response time (RT) at one movement of the sequence (other than the 1st) was taken as evidence for the operation of a further process of sequence organization after initialization. We refer to such organizational features of sequences as “chunk points.”

In experiments 2–4, we sought to capitalize on the temporal specificity of transcranial magnetic stimulation (TMS) to investigate the role of the human pre-SMA at specific time points within a well-learned sequence. Single pulses or short trains of TMS transiently disrupt the normal pattern of activity in a cortical area for tens or hundreds of milliseconds, and behavioral performance is also disrupted and slowed if the brain area is essential for task performance (Jahanshahi and Rothwell 2000; Pascual-Leone et al. 2000; Walsh and Cowey 2000; Walsh and Pascual-Leone 2003). Experiment 2 was designed in the same way as experiment 1, but now trains of 10-Hz repetitive TMS (rTMS) were applied over the pre-SMA just prior to the first movement, the chunk point, and the nonchunk point (a movement in the middle of a preorganized chunk). We predicted that the TMS would disrupt performance if applied when the sequence was initially organized (1st movement) or subsequently updated (chunk point) but not when it was ap-

**FIG. 1.** Subjects sat facing a computer monitor and made finger press sequences using an 8-key button press box where the buttons are numbered from left to right, with the left little finger button 1, the left index finger button 4, the right index finger button 5 and the right little finger button 8. Initially, subjects were taught these sequences using visual guidance (left). Nonmoving fingers were indicated by blue ellipses while a red ellipse instructed the proper move. The visual guidance of each movement continued for the entire sequence on a given trial. If a mistake was made during the sequence, a 100-Hz, 200-ms tone was sounded and the trial was stopped. The red ellipse remained to indicate the proper move and a green ellipse appeared over the finger position that the subject actually pressed. In trials 5–20, visual guidance was pseudorandomly removed on some of the trials so that the subjects only saw a blank screen and had to perform the sequence from memory (memory guided) without visual guidance (right). All trials were memory guided after trial 20. Once subjects successfully completed 10 consecutive memory guided trials without error, experimental trials began.
plied at the nonchunk point. Experiments 3 and 4 (Fig. 2) further explored the effect of pre-SMA TMS on the first movement of a sequence when subjects performed two short sequences with visual cues instructing which sequence to perform (experiment 3, cue-sequence task) or when visual cues instructed subjects to either repeat or switch sequences (experiment 4, cue-change task). To test the specificity of any disruptive effects of pre-SMA TMS, we also examined the effect of TMS applied over the dorsal premotor cortex (PMd). The control experiments focused on the left PMd because it has been shown to be dominant for movement selection (Johansen-Berg et al. 2002; Schluter et al. 1998).

**Methods**

**General task**

All of the experiments consisted of bimanual sequences of finger movements using an eight-button keypad, where the fingers are numbered from left to right starting with the left little finger as key 1 and the right little finger as key 8 (Fig. 1). Subjects nearly always alternated hands with each movement of a sequence, and each sequence was carefully designed to prevent statistical or relational structure patterns that may impose artificial sequence organization, i.e., runs, repetitions within consecutive or adjacent movements, transpositions, inversions, or mirroring of movements (see Keele et al. 1990; Koch and Hoffmann 2000 for reviews). An example of such statistical or relational structure would be a sequence of twelve movements such as 123-123-234-432, where elements 1–3 are a run; elements 4–6 are a repetition of elements 1–3; elements 7–9 are a transposition of elements 4–6; and elements 10–12 are an inversion of elements 7–9. Bimanual sequences were used (as opposed to unimanual sequences) to reduce statistical or relational structure patterns that could influence the subject group to chunk at a specific position in the sequence. To keep from introducing any new variables, bimanual sequences were also used in the investigations of sequence initiation in experiments 3 and 4.

Subjects were seated facing a PC computer with their chin in an adjustable chin rest. Subjects were first given specific instruction about the task. Once the task had been explained, each subject was given a learning block. Sequence learning was initially aided by visual guidance (Fig. 1, left). Each trial of a sequence began with a 500-Hz warning tone that lasted 500 ms, followed by a 1-s delay before a 150-ms initiation tone was sounded. The eight different finger positions corresponding to the orientation of the hands on the eight-button keypad were then displayed on a colored screen. Blue ellipses defined seven of the finger positions and a red ellipse marked the finger to be pressed. After the subject pressed the appropriate finger, a 500-Hz, 200-ms correct tone was sounded. A blue ellipse then replaced the red ellipse, and a red ellipse appeared at a new finger position instructing the next movement of the sequence. This continued until the sequence was completed. Each complete sequence defined a trial. Response times for the first movement in the sequence were recorded from the illumination of the screen beginning the trial. Response times (or inter-response times) for movements after the first movement were recorded from the end of the previous movement’s correct tone. If a mistake was made during the sequence, a 100-Hz, 200-ms error tone was sounded, and the trial was aborted. The red ellipse remained to indicate the proper move, and a green ellipse appeared over the finger position that the subject actually pressed. The first five learning trials were aided by visual guidance. In trials 5–20, visual guidance was pseudorandomly removed on some of the trials so that the subjects only saw a blank colored screen and had to perform the sequence from memory (memory guided) without visual guidance (Fig. 1, right). After learning trial 20, all of the trials were memory guided until the...
subjects could reach the criterion of 10 consecutive memory-guided trials without an error. This criterion was reached, the training block ended and the subjects began memory-guided experimental blocks.

TMS procedures

All TMS pulses were generated by a Magstim Rapid TMS machine (Whitland, Whales, UK), with a maximum output of 2.0 T. The parameters used were within the previously established safety guidelines (Wassermann 1998) and were approved by the Central Oxfordshire Research Ethics Committee (Reference No. C99.178). All subjects were right-handed, healthy individuals with no history of head injury or seizure history within the family. Informed consent was obtained from all participants before participation.

PRE-SMA. We localized the pre-SMA site using a method previously reported (Hadland et al. 2001; Rushworth et al. 2002). We first localized the foot representation within each subject’s motor cortex by using single-pulse stimulation along the midline starting at a point 2 cm posterior to Cz. Coil position was then adjusted in steps of 0.5 cm as the stimulation intensity was once again reduced to determine the maximally excitatory foot representation in the motor cortex. The coil was then moved 6 cm anterior to stimulate the pre-SMA.

In previous studies, magnetic resonance image (MRI)-guided frameless stereotactic confirmation of coil position had shown that moving the coil 5–6 cm anterior to the motor cortex placed it above the pre-SMA region (Hadland et al. 2001; Paus 1999; Rushworth et al. 2002). In the present study, coil positioning above the pre-SMA was confirmed by using the BrainSight frameless stereotax system (Rogue Research, Montreal Canada) for 10 subjects (12 localizations in total, see Fig. 3, left). For these subjects, a Polaris (Northern Digital, Waterloo, Canada) infra-red tracking system was used to measure the position of anatomical landmarks on each subject’s head that are also visible on each MRI scan (nose tip, bridge of nose, left and right infra-tragal notches). Each subject’s head and MRI were then co-registered, and another infra-red tracker was used to monitor the position of the TMS coil with respect to the brain. Each subject’s brain MRI was also registered to an average of 305 brains aligned with Talairach space (Collins et al. 1994; Talairach and Tournoux 1988), using Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library tools (FMRI, Oxford, UK; www.fmrib.ox.ac.uk/fs/1) so that TMS stimulation coordinates could be identified in standard space (Fig. 3, left). This method confirmed that in 10 of 12 cases subjects received TMS over the midline at or anterior to the vertical line (VAC line) through the anterior commissure at a position between \( y = -2 \) mm and \( y = 23 \) mm (group mean, \( y = 5.5 \) mm) at the approximate position of the pre-SMA (Picard and Strick 1996, 2001; Stephan et al. 1995; Vorobiev et al. 1998). There was a slight tendency for the TMS site to be biased toward the left hemisphere (group mean coordinate \( x = -5.1 \), shown as slice \( x = -7 \) in Fig. 3, left). This may reflect a lateral bias introduced while localizing the foot motor hot spot, which tends to be just off midline.

Our previous experiments have all suggested dominant roles for left hemisphere motor areas (Johansen-Berg et al. 2002; Rushworth et al. 2001, 2002; Schluter et al. 1998).

All pre-SMA TMS experiments used 10-Hz, 5-pulse rTMS trains at 110% of each subject’s individually determined active motor threshold. Stimulation was applied with a Magstim “cone” coil in a similar manner to previous TMS investigations of medial frontal cortex (Hadland et al. 2001; Harmer et al. 2001; Rushworth et al. 2002). PMd. In the series of control experiments, TMS was directed over the left PMd. The PMd site was localized using a method previously reported (Johansen-Berg et al. 2002; Praamstra et al. 1999; Rushworth et al. 2002; Schluter et al. 1998, 1999). We first localized the hand representation within each subject’s motor cortex by using single pulse stimulation starting at a point 2 cm anterior and 4 cm lateral to Cz. The stimulation strength began at 40% of stimulator output and was increased in steps of 5% until a just visible twitch was seen in the right hand while subjects were instructed to spread their fingers using 10% of maximum force. Coil position was then adjusted in steps of 0.5 cm as the stimulation intensity was once again reduced to determine the hot spot.

The coil was then moved 2 cm anterior and 2 cm medial to the motor cortex hot spot to stimulate PMd. The coil was adjusted to a slightly more anterior and medial position if TMS pulse trains at experimental parameter levels caused visible motor twitches. In previous studies, MRI-guided frameless stereotactic confirmation of coil position had shown that moving the coil to this position placed it above the superior branch of the superior precentral sulcus in the approximate vicinity of PMd (Johansen-Berg et al. 2002; Paus 1999; Rushworth et al. 2002). In the present study, coil positioning over the PMd was confirmed for 12 subjects (15 localizations in total, see Fig. 3, right) by using MRI guided frameless stereotactic localization as described in the preceding text. Coil placement over the PMd was confirmed (group mean: \( x = -28.7 \) mm, \( y = 6.8 \) mm, \( z = 67.0 \) mm).

All PMd TMS experiments used 10-Hz, 5-pulse rTMS trains at 110% of each subject’s individually determined active motor threshold to elicit a twitch of the fingers. Stimulation was applied with a Magstim 70 mm “figure 8” coil in a similar manner to previous TMS investigations of PMd (Johansen-Berg et al. 2002; Praamstra et al. 1999; Rushworth et al. 2002; Schluter et al. 1998, 1999).

Subjects

In total, 32 subjects participated in the series of four experiments that were conducted over an 18-mo period. Testing was not completed for one subject, who found the PMd TMS uncomfortable, and there was insufficient data for analysis. In total, 54 subject data sessions were gathered and form the basis of this report. Some subjects participated in more than one experiment. For this reason, the sequence changed between TMS sites and between experiments to avoid practice effects. Thus within an experiment, the sequence in a pre-SMA group remained the same for all subjects but differed from the sequence performed by the PMd group. No more than two subjects
ever participated as subjects within both TMS groups of the same experiment, and subjects never received TMS at both sites within the same testing session. Although the order of experiments was not randomized (the design of later experiments was determined by the results of the earlier experiments), there were long intervals of weeks or months between experiments while results were analyzed, new tasks were designed, and subjects had MRI scans. Details of each sequence are given in the following text.

All subjects were naïve to the purpose of the chunking experiments, and no indication was given to the subject that organizational strategy in movement was the focus of the study. Two of the authors took part as subjects in experiment 3 and one of the authors participated in experiment 4. Analyses of the data both with and without the authors’ data always lead to similar outcomes.

Analysis

TMS disruption is transient, and RT increases, rather than error increases, have proven to be sensitive indices of behavioral performance (Bestmann et al. 2002; Campana et al. 2002; Johansen-Berg et al. 2002; Praamstra et al. 1999; Rushworth et al. 2002; Schluter et al. 1998, 1999; Walsh and Cowey 1998; Walsh et al. 1998). Therefore as in previous TMS experiments, analysis focused on RT increases. However, because high-frequency TMS over the more caudal medial premotor cortex has previously been shown to induce sequencing errors (Gerloff et al. 1997), an analysis of error rates was performed, but TMS did not significantly influence error rates in any of the present experiments, and therefore error rates will not be reported. For simplicity, the term “RT” will be used as a convention, even when referring to response times of movements after the first movement. For each subject, we calculated the median RT and error rate for each sequence position for all of the repetitions of that sequence. These median scores for each movement position and each subject were then analyzed using repeated-measures general linear models (GLMs), using within-subject correction procedures (Huynh-Feldt) where appropriate. A group mean of the individual median RTs or error rates for each subject was then calculated and reported in all cases unless otherwise specified. When necessary, the interpretation of significant interaction effects was aided by one tailed paired sample t-test.

Experiment 1: behavioral chunking

This experiment was performed to examine how subjects represent long sequences of actions and to see whether there is a tendency to divide a long sequence of movements into smaller component chunks.

SUBJECTS. Eight subjects performed this task (6 female, 2 male). Subjects were between 21 and 25 yr of age and all were right handed.

TASK. Subjects performed a bimanual sequence of 12 finger movements (617428362753). Subjects were first trained on the sequence as previously described. Once criterion was reached during the training block, three blocks of 20 memory-guided trials were performed with an inter-trial interval of 4.5 s. Subjects were cued to initiate a trial by the appearance of a yellow screen and an 800-Hz initiation tone. During the inter-trial interval, subjects saw only a black screen. Once all three experimental blocks were finished, the data were examined for the existence of chunking.

Experiment 2: chunking task with TMS applied over the pre-SMA or PMd

The main objective of this experiment was to examine whether 10-Hz rTMS over the pre-SMA or left PMd disrupted the performance of a sequential button-pressing task when applied at different time points during the sequence. Three stimulation points were examined: the first movement of the sequence; the “chunk point,” the movement with the highest RT within the sequence indicative of the initiation of a new chunk; and at the nonchunk point, a low-RT movement in the middle of a chunk.

SUBJECTS. Fourteen subjects performed this task (11 male, 3 female; 7 subjects in each TMS group). Subjects were between 23 and 40 yr of age and all were right handed.

TASK. The experimental stimuli and design were the same as in experiment 1, but the actual sequences differed (pre-SMA group: 257361742835; PMd group: 471526382746). Subjects were first trained on the sequence as previously described. Once criterion was reached during the training block, a memory-guided behavioral block of 20 trials was performed. This block was used to assess the location of each subject’s chunk point and nonchunk point on the basis of an immediate median RT analysis. The chunk and nonchunk points were always separated by at least three other intervening movements and the nonchunk point could never be the last movement of the sequence. The task program was then adjusted so that TMS trains could be administered according to a pseudorandom design on 35% of the trials of the first movement of the sequence, the chunk point movement, and the nonchunk point movement. Each subject then performed two blocks of 30 memory-guided trials with TMS applied at any of the three stimulation points. TMS at the first movement point occurred at the offset of the 800-Hz initiation tone. TMS at both the chunk or nonchunk points occurred immediately after the subject completed the movement preceding either point.

Experiment 3: cue–sequence task with TMS applied over the pre-SMA or PMd

This experiment was designed to examine whether the first movement of a sequence might be more susceptible to TMS induced disruption if subjects were required to switch between two short sequences. Subjects were taught two sequences, and on each trial they were instructed to initiate a sequence by the presentation of one of two easily identifiable sensory cues.

SUBJECTS. Fourteen subjects performed this task (12 male, 2 female; 8 subjects in pre-SMA group, 6 subjects in PMd group). Subjects were between 19 and 36 yr of age and all were right handed. Three subjects (2 male, 1 female, ages 23–34), all right handed, performed a second control experiment.

TASK. Subjects performed two bimanual sequences of five nonrepeating finger movements. Subjects were taught two cue–sequence associations. They were told to perform sequence A (pre-SMA group: 73516; PMd group: 61738) when they saw a pink background and heard a 200-Hz initiation tone and to perform sequence B (pre-SMA group: 38462; PMd group: 46283) when they saw a yellow background and heard an 800-Hz initiation tone. Subjects were then given a practice block with both sequences randomized over the block using the learning criterion previously described. After completion of 10 consecutive trials without error, the training block was terminated and two blocks of 30 memory-guided trials were performed. The two different sequences were pseudorandomly generated across these two blocks with TMS pseudorandomly applied on 50% of the trials at the offset of the initiation tone. In a subsequent control experiment to investigate the importance of the precise onset of the 500-ms TMS train, we applied the first pulse of TMS over the pre-SMA at the onset of the initiation tone.

Experiment 4: cue-change task with TMS applied over the pre-SMA or PMd

The cue–sequence task used in experiment 3 was intended to employ an association between cues and sequences. There were also, however, inadvertent associations between each cue and the first movement of each sequence. For example, not only was the pink screen color and low pitched tone associated with sequence A, but it...
was also consistently associated with the first movement of sequence A, whereas the yellow screen color and high pitched tone was not only associated with sequence B, but also with the first movement of sequence B. Experiment 4 was designed to examine the effect of TMS on the retrieval of movement sequences in the absence of the confounding effect of cue-first movement associations. In experiment 4, subjects were once again taught two sequences, and once again they were required to alternate between them. Now, however, the visual cues presented at the beginning of each trial simply instructed subjects to repeat the previous sequence or to switch to the other sequence. In this way, it was ensured that no simple cue-first movement associations could operate. As is shown in the following text, the change in behavioral context clearly altered the way the task was performed and the sequence initiation RTs were affected. To approximately equate the average movement initiation RT in the present experiment with that used in experiment 3, we taught subjects a slightly longer bimanual sequence of six movements.

**SUBJECTS.** Fifteen subjects performed this task (13 male, 2 female; 8 subjects in pre-SMA group, 7 subjects in PMd group). Subjects were between 22 and 33 yr of age and all were right handed.

**TASK.** Subjects performed two bimanual sequences of six nonrepeating finger movements (pre-SMA group: 284637, 527163; PMd group: 351726, 735284). The experimental stimuli were similar to the previous experiments. Subjects were told they were to perform two different sequences and that whenever they saw a pink screen, they were to repeat the same sequence as they had performed on the previous trial, and whenever they saw a yellow screen, they were to switch to the other sequence they had learned (Fig. 2). Switch trials occurred every four to seven trials, although subjects were only told they would never have to switch on successive trials.

Subjects were first trained on each sequence separately. The screen color for each of the sequences during training was blue. Once they could perform each sequence to criterion, they were given a practice block of the experimental condition. Both the practice and experimental blocks began with visual guidance of the same sequence for the first three trials. Because there were no sensory cues to guide sequence selection, visual guidance was needed at the very beginning of a block to inform the subjects which sequence to continue performing (Fig. 2, bottom). On trial 4, the visual guidance disappeared, and subjects only saw a pink screen and had to recall which sequence they performed on the previous trial to select the correct response on the current trial. Once a yellow screen appeared, subjects were required to switch to the other sequence. After completion of 10 consecutive trials without error, the training block was terminated and an experimental block was performed. Each trial was initiated with an 800-Hz, 150-ms tone, and the inter-trial interval was 3.5 s. TMS was pseudorandomly applied on 50% of the switch trials and 20% of the repeat trials. Because of the pseudorandom design, each subject performed enough trials to get ≥10 data points for each of the four possible conditions: TMS-switch, no TMS-switch, TMS-repeat, no TMS-repeat. This meant the number of trials each subject performed varied between 100 and 200 trials for each of the TMS locations. TMS was applied at the onset of the initiation tone.

**RESULTS**

**Experiment 1: behavioral chunking**

The performance of an example subject in each of the three blocks of 20 repetitions of the sequence is shown in Fig. 4. The first movement of the sequence is characterized by a long RT that indexes the planning and organizing of the upcoming sequence rather than the difficulty of selecting a particular movement (Sternberg et al. 1978). There is, however, an additional clear increase in RT on the eighth movement indicating the updating and reorganization of the sequence at this point. The sequence is divided into two chunks of seven and five movements by this subject, and this pattern of chunking appears to be stable throughout three testing blocks. The long RT on the eighth movement is an example of what we refer to as a chunking point. This subject’s chunk point was, on average, 229 ms slower than the subject’s next slowest movement.

When all subjects’ data were averaged, there was a suggestion (Fig. 5A) of a general increase in RT on movements seven through nine, but a clear and uniform chunk point was difficult to discern at the group level. It was possible, however, to re-plot the data with respect to the longest RT for each subject (Fig. 5B). For example, the data for the individual subject shown in Fig. 4 would be re-plotted with respect to the eighth movement, which would be labeled point 0. The 9th and 10th movements would be re-labeled points 1 and 2, whereas the 6th and 7th movements would be re-labeled points −2 and −1, respectively. As expected, inspection of Fig. 5 (right) reveals the re-emergence of a chunk point at the group level after this reordering process. The RTs at the different points are now significantly different from one another $[F(3.01,15.06) = 4.97, P = 0.014]$ and the chunk point RT at point 0 is significantly longer than either of the adjacent RTs ($P < 0.05$). Importantly each subject’s re-ordering protocol from the first block (Fig. 5, A and B) can be applied to data gathered in the second block (Fig. 5C) or third block (Fig. 5E) and the chunk point re-emerges (Fig. 5, D and F). Again in each case, the chunk point RT in each set is significantly longer than either of the adjacent RTs. In brief, although the chunking point varied between subjects, it remained at a consistent position within the sequence for a given subject.

Closer inspection of the data suggested clear evidence of chunking in six of the eight subjects (at mean sequence posi-
For these six subjects, the chunk point RTs were on average 245 ± 70 (SE) ms slower than the next slowest movement once the first movement was excluded. The evidence for one subject appeared more equivocal. In one case, there was a suggestion of two chunk points as if the sequence were being divided into three chunks.

**Experiment 2: chunking task and the effects of pre-SMA or PMd TMS**

The subjects demonstrated clear and similar chunking effects during the behavioral sessions in both the pre-SMA and PMd experiments. The mean chunk point RTs were 681 ± 145
The data were analyzed with a three factor GLM (3 levels of point: 1st movement, chunk point, nonchunk point; 2 levels of TMS: control, TMS; 2 levels of group: pre-SMA, PMd). The data for one subject who had participated in both pre-SMA and PMd groups were removed so that a between subject approach could be used to compare the two TMS groups. The absence of any main effect of group (P > 0.1) suggested that both groups were performing the sequences in a similar way. Likewise an analysis of just the baseline non-TMS trials confirmed the similarity of the performances of the two groups; again there was no main effect of group (P > 0.1). There was a two-way quadratic interaction between point and TMS [F(1,10) = 9.65, P = 0.011], consistent with the TMS having a different effect when it was delivered at one of the three points within the sequence. Moreover, the effect was modulated by the site of TMS; there was a three-way quadratic interaction among point, TMS, and group [F(1,10) = 10.11, P = 0.010]. Subsequent one-tailed linear contrasts showed that TMS had a significantly different effect when it was delivered at the chunk point depending on whether it was delivered over the pre-SMA or the PMd [F(1,10) = 3.80, P = 0.040] but not when it was delivered at the time of the first movement or the nonchunk point (P > 0.1). The analyses suggest that TMS only had a disruptive effect when it was delivered over the pre-SMA and only when it was delivered at a chunk point.

The interpretation was confirmed by a subsequent analysis of just the data from the pre-SMA group. There was a significant interaction between movement position and TMS [F(1.92,11.50) = 4.03, P = 0.048], suggesting that the disruptive effect of pre-SMA TMS depended on the time of its application. Pre-SMA TMS significantly slowed RT at the chunk point [t(6) = 2.16, P = 0.038], from a mean of 290–358 ms but not when it was applied at the first movement or the nonchunk point (Fig. 6). No such effects were apparent in a separate analysis of PMd TMS (Fig. 7).

It might be argued that the temporal relationship between pre-SMA TMS and movement is confounded when the chunk and nonchunk points are compared; by definition, the chunk point movement has a longer RT than the nonchunk point movement and the pulse train might not have finished before the nonchunk movement was made. The movements that follow the designated nonchunk movement, however, do stand in the same temporal relationship to the TMS as is the case for chunk movements. We therefore examined the effect of pre-SMA TMS on the first and second movements after the nonchunk movement. Not only did pre-SMA TMS not have any significant disruptive effect at the nonchunk point [t(6) = 0.36, P > 0.05], but it also had no significant disruptive effect on either the first [t(6) = 0.92, P > 0.05] or the second [t(5) = 1.17, P > 0.05] movement after the nonchunk movement.

Experiment 3: cue-sequence task and the effects of pre-SMA or PMd TMS

The data were analyzed with a three factor GLM (2 levels of switch: switch sequence vs. repeat sequence; 2 levels of TMS: TMS vs. control non-TMS; 2 levels of group: pre-SMA vs. PMd group). The two subjects who had participated in both TMS groups of experiment 3 were removed from the pre-SMA group to maintain the highest statistical power. The absence of any main effect of group (P > 0.1) suggested that both groups were performing the sequences in a similar way. Likewise an analysis of just the baseline non-TMS trials confirmed the similarity of the performances of the two groups; again there was no main effect of group (P > 0.1).

The analyses, however, revealed no differences in the effect of pre-SMA or PMd TMS on the first movement RT. Subsequent analysis of just the pre-SMA data (Fig. 8A) or just the PMd data (Fig. 8B) confirmed the absence of any effect of TMS on first movement RT.
It was noted in both pre-SMA and PMd groups that, surprisingly, subjects showed no RT cost of having to switch sequence.

Although TMS did not disrupt performance of the first movement, it was apparent that pre-SMA TMS slowed RTs on subsequent movements (Fig. 9A). A post hoc analysis of the individual RTs after the first movement (movements 2–5) revealed a significant slowing of RT when pre-SMA TMS was applied \( [F(1,7) = 8.52, P = 0.022] \). By summing the individual RTs for each sequence, a measure of total sequence time (TST) was calculated. Despite the lack of a pre-SMA TMS effect on the first movement, TST (Fig. 9A) was significantly slowed on pre-SMA TMS trials \( [F(1,) = 65.29, P < 0.001] \). As can be seen in Fig. 9A, pre-SMA TMS application slowed total sequence time by 91 ms on switch trials and 211 ms on repeat trials. Such a pattern of results suggested that the first movement was somehow preserved from the disruptive effect of the pre-SMA TMS, whereas future movements were not. No such effects were observed in a similar post hoc analysis of the PMd data. (Fig. 9B). Two possible interpretations of the pre-SMA TMS effect were tested. First, we investigated whether the TMS was being applied slightly too late to affect the first movement. In a control experiment, we applied the first pulse of the 500-ms pre-SMA TMS train 150 ms earlier, at the onset rather than the offset, of the initiation tone. Even in a small group of three subjects the same pattern of results was clear. Pre-SMA TMS never caused any disruption on the first movement (pre-SMA TMS made all 3 subjects faster on both switch and repeat trials) of the sequence, but the individual RTs of subsequent movements (movements 2–5) were significantly slowed \( [F(1,2) = 57.17, P = 0.017] \). The second possibility, that the type of cueing used to instruct the sequences was the critical determinant of whether a first movement deficit would be found, was tested in experiment 4.

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** First movement RT data for the cue-sequence task in experiment 3. Pre-SMA TMS (A) or PMd TMS (B) did not disrupt the initiation of a sequence.

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Total sequence time data for the cue-sequence task in experiment 3. A: pre-SMA TMS significantly slowed \( (*P < 0.05) \) the total time required to complete the sequence. B: PMd TMS did not significantly disrupt total sequence time.
Experiment 4: cue-change task and the effects of pre-SMA or PMd TMS

A between-subject GLM analysis, similar to that used in experiment 3, was conducted on the first movement RTs. The two subjects who had participated in both TMS groups of experiment 4 were each assigned to just one group. Unlike in experiment 3, there was a significant main effect of switch \(F(1,11) = 43.86, P < 0.001\), a significant interaction between TMS and switch \(F(1,11) = 5.17, P = 0.044\), and a three-way interaction among TMS, switch, and whether subjects were in the pre-SMA or PMd group \(F(1,11) = 6.06, P = 0.032\).

A separate analysis of just the pre-SMA data (Fig. 10A) confirmed that pre-SMA TMS significantly slowed RT on the first movement \(F(1,7) = 7.94, P = 0.026\). Control RTs increased from a mean of 445 ms when the sequence repeated to a mean of 810 ms when the sequence switched. When TMS was delivered, the RTs increased to 580 and 942 ms, respectively. Such an analysis of just the PMd data (Fig. 10B) did not suggest that PMd TMS had the same disruptive effect. There was an interaction between the effect of TMS and the switching of sequence \(F(1,6) = 8.10, P = 0.029\), but subsequent analysis revealed no indication of a significant slowing in the RT of the first movement with TMS application. Instead the interaction was due to subjects being faster on switch trials when PMd TMS was applied (874 ms) as opposed to switch trials without TMS application (1,046 ms). On repeat trials, subjects were nonsignificantly slower on TMS trials (661 ms) than on non-TMS trials (568 ms).

The total sequence times (TSTs) were also measured as in experiment 3. Because TMS was associated with an outlying, very high TST for one subject in the pre-SMA group (3,905 ms as opposed to a mean of 1,303 ms for the remaining 7 subjects), a logarithmic transformation was applied to the data prior to analysis. There was a significant disruptive effect of TMS \(F(1,11) = 6.44, P = 0.028\) and a significant effect of switching \(F(1,11) = 50.84, P < 0.001\) on TST, but no TMS by group interaction was present \(P > 0.1\). However, further analysis revealed that the disruptive effect of TMS on TST was significant \(F(1,7) = 7.97, P = 0.026\) in the pre-SMA group (Fig. 11A) but not in the PMd group \((P > 0.1\), Fig. 11B\). When pre-SMA TMS was applied, TSTs increased from 836 and 1,365 to 1,042 and 1,629 ms on repeat and switch trials, respectively.

Re-analysis of chunking data

After obtaining three sets of data from the chunking tasks (experiments 1 and 2), it was possible to carry out a more extensive analysis to identify factors that might have influenced chunking. We categorized each possible movement or movement transition in terms of the hand used (dominant, right hand or subdominant, left hand), the finger used (index, middle, or ring: there were insufficient little finger movements for analysis), and the distances between consecutive movements (the movement transitions were divided into 2 approximately equal-sized categories of near and far distances in which consecutive movements were 2–4 or 5–6 finger positions apart). Pearson’s \(\chi^2\) tests were then used to test if the observed frequencies of chunking in each type of category differed from the expected frequencies. There was no significant effect of hand \(\chi^2(1, n = 20) = 0; P > 0.05\), finger \(\chi^2(2, n = 20) = 1.82; P > 0.05\), or inter-movement distance \(\chi^2(1, n = 20) = 0; P > 0.05\). Chunking was not simply just associated with movements that involved transitions between the two hands. The hand used changed with nearly every movement of each sequence, but chunking patterns remained stable while a subject performed a sequence. The expected frequency of chunking in the absence of a hand transition was, therefore, too low to be analyzed. It was, nevertheless, noted that the observed and expected frequencies of chunking were consistent when movements were categorized on the basis of hand transition.

DISCUSSION

Organization of movement sequences into chunks

Experiment 1 demonstrated that subjects exhibit a spontaneous tendency to organize long sequences of actions into component chunks. As previously noted (Sternberg et al. 1978), the
RT of the first movement of a sequence tended to be longer than subsequent RTs. It has been argued that the long RT does not reflect the difficulty of selecting the first movement of the sequence, but rather it is an index of retrieval and preparation of the whole sequence of movements. Later in the sequence, between approximately the seventh and ninth positions in the sequence, there was a second increase in RT of, on average, 243 ms compared with the next slowest movement (other than the very 1st movement). We refer to this movement as the chunk point because the sequence appears to be divided into two component chunks at this position. Although the chunk point occurred at a constant position for any given subject throughout testing, its position varied between subjects (see Figs. 4 and 5). Hand dominance, transitions between hands, distances between the fingers used in consecutive movements, and finger identity did not provide any simple account of chunking patterns. Although it may be possible for such factors to influence chunking, they did not do so in the present experiments where nearly every movement transition involved an alternation of the hand. Such a variety of chunking patterns suggests that the chunking phenomenon is not simply the consequence of a particular movement transition that is physically difficult for all subjects to perform but rather reflects an organizing principle for the performance of a sustained motor program; part way through the sequence there appears to be a need to update the organization of the sequence and to retrieve the final chunk of movements. The timing of the chunk point, at approximately the seventh to ninth movement of the sequence, suggests that it may be related to the limited capacity of short-term memory stores (Miller 1956).

There have been previous claims of structured representations in movement sequences but these have tended to depend on presenting subjects with highly structured sequence instructions and then observing the degree to which the instructed structure is preserved during performance (Keele et al. 1990; Koch and Hoffmann 2000; Nissen and Bullemer 1987; Povel and Collard 1982; Rosenbaum et al. 1983). For example, subjects have been taught short three movement sequences and then longer sequences that could be decomposed into the previously taught short sequences (Verwey 2001). Long RTs have been observed at the transition points between the structured components in such experiments. The structured representation of the sequence in the present experiment emerged spontaneously in the absence of any specific instruction or artificial imposition (runs, repetitions, inversions or transpositions) and in a slightly varying way for each subject. Moreover similar results were observed in experiment 2, with different sequences, when the effect of TMS was examined in the context of chunking (Figs. 6 and 7).

**Role of the pre-SMA in updating sequence chunks**

The role of the pre-SMA in updating sequence chunks was tested in experiment 2 by the application of 0.5-s 10-Hz rTMS trains at either the chunk point or in the middle of a chunk at a position referred to as the nonchunk point. As predicted, pre-SMA rTMS disrupted performance, causing significantly longer RTs when it was applied at the chunk point at the initiation of a new sequence chunk but not when it was applied during the course of an ongoing chunk at the nonchunk position (Fig. 6). No disruptive effect of TMS was seen when it was applied over PMd, a control site also within the motor system (Fig. 7). This is consistent with the results of previous studies that have suggested a lesser involvement of the more lateral premotor cortex in action sequences (Chen et al. 1995; MUSHIAKE et al. 1991).

It might be argued that the absence of a TMS effect at the nonchunk point is due to it taking some time for the effect of the TMS to accumulate and become disruptive, and RTs were, by definition, shorter at the nonchunk position. Because the nonchunk point had a control RT of 160 ms and the TMS train would not have been completed by this time, we examined the effect of TMS on the following two movements. Post hoc tests confirmed that no disruption to the two movements after the nonchunk point was induced by pre-SMA TMS. Moreover, a comparison of Figs. 6, 8, and 10 demonstrates that the susceptibility of a sequence’s initial movement to being disrupted by pre-SMA TMS was not a function of its RT. Initial sequence
movements with comparatively short RTs (445 ms) in experiment 4 were disrupted by pre-SMA TMS, whereas initial sequence movements with longer RTs in experiments 2 and 3 (579 and 695 ms) were unaffected by pre-SMA TMS. In summary, the application of pre-SMA TMS at the chunk point caused an immediate disruption to performance, but it did not disrupt performance at the nonchunk point or any of the movements that immediately followed the nonchunk point.

Hikosaka and colleagues (Hikosaka et al. 1995; Nakamura et al. 1998; Rand et al. 1998, 2000) taught their monkeys long sequences of 10 movements by teaching them series of five short 2-movement sequences or sets. They referred to the entire 10-movement sequence as a hyperset. It is possible that each set within the hyperset could be likened to a chunk in the present experiment. Most of the neurons in the pre-SMA that were preferentially active in relation to the new learning of sequences were active on just the first movement of each set. On well-learned hypsets, there was little pre-SMA activity apart from the first trial or even first set at a time when the monkey had to discard one hyperset and retrieve and organize a different hyperset (Nakamura et al. 1998). The pattern of behavioral interference when TMS was applied at chunk points, but not at nonchunk points, might be predicted from the intermittent pattern of pre-SMA single neuron activity during the course of sequence performance.

At the chunk point, subjects have to update their current action plans. Matsuzaka and Tanji (1996) have reported activity in the pre-SMA specifically related to the changing of an action plan. They trained monkeys to respond to one of two targets and found 31% of pre-SMA neurons were active when the response involved a shift away from the movement made on the previous trial. Within the medial frontal cortex such responses were most prominent in the pre-SMA rather than in the SMA. Shima et al. (1996) taught monkeys several three-movement sequences. The monkeys performed a given sequence for 11 repetitions and were then guided through performance of another sequence. They reported that 25% of pre-SMA neurons were most active at the transition between one sequence and the next. It could be argued that the transition between one movement chunk and another in the present experiment involves a similar change in current action plans.

The chunk sizes in the present experiment (mean sequence position: 7.14 ± 0.67) seem to reflect the constraints of working memory capacity for a limited number of items (Miller 1956). The anatomical connections of the pre-SMA mean that it is ideally situated to retrieve and update motor plans as working memory is, in turn, updated. The pre-SMA has reciprocal connections with the prefrontal cortex (Bates and Goldman-Rakic 1993; Lu et al. 1994; Luppino et al. 1993), which has been implicated in working memory (Goldman and Rosvold 1970), as well as connections with the SMA, which projects to M1 and the spinal cord (Dum and Strick 1991, 1996; He et al. 1995). BOLD signal responses in the pre-SMA are modulated by working memory demands even if disruption of the area does not produce straightforward working memory deficits (Hadland et al. 2001). Both the pre-SMA and the prefrontal cortex are active when subjects are learning new motor tasks (Gomez Beldarrain et al. 2002; Jueptner et al. 1997; Nakamura et al. 1998; Robertson et al. 2001; Sakai et al. 1998, 2002; Toni et al. 1998), although of these two structures, the prefrontal cortex may have a dominant role in implicit procedural learning (Pascual-Leone et al. 1996; Willingham et al. 2002).

Activation within the prefrontal-pre-SMA circuit during learning might reflect the organization and refinement of the task into manageable chunks. Bor et al. (2003) have shown that lateral prefrontal activity increases when it is possible to organize task information into chunks. Anterior premotor regions, such as the pre-SMA and anterior parts of PMd, may then be important when the information in working memory is translated into a program for action (Ohbayashi et al. 2003).

Role of the pre-SMA in first initiating a sequence of movements

Contrary to initial predictions, pre-SMA TMS had no disruptive effect when it was applied before the very first movement in the sequence in experiment 2 when the first sequence chunk was retrieved and prepared. Although many pre-SMA neurons are responsive during the new learning of sequences, those that remain active after learning tend to fire at the beginning of the sequence (Nakamura et al. 1998). A further series of experiments (3 and 4), however, demonstrated that pre-SMA TMS did disrupt the initiation of a sequence but that this disruption could only be detected after removing the influence of confounding variables as discussed in the following text. Control experiments demonstrated that TMS over PMd never affected sequence initiation.

There are several reasons for thinking that the first movement in experiments 2 and 3 might have been particularly resistant to disruption. The resistance of the first movement to disruption might have been related to over familiarity with the very first movement of the sequence. The trial-and-error learning procedure used in teaching the sequences might have meant that subjects were especially familiar with the first movement in comparison with the subsequent movements. Moreover, the first movement to be performed at the beginning of every trial had always remained constant for each subject in experiment 2. In the cue-sequence paradigm (experiment 3), subjects were taught two different five-movement sequences, each associated with specific visual and auditory cues (Fig. 2) so that the first movement of each trial could be one of two possible movements. Once again no disruptive effect of TMS on the first movement was observed.

A second reason for the resistance of the first movement in a sequence to TMS disruption in experiments 2 and 3 was also considered. The sequence onset was cued by a change in screen color and tone. These sensory cues might have functioned not only as a cue to retrieve the sequence but also as a cue to specifically select the first movement of the sequence. Even in experiment 3 it would have been possible for such associations between cues and individual movements to operate because the yellow screen and high tone were always associated with one first movement, whereas the pink screen and low tone were always associated with another first movement independently of the retrieval of each of the whole sequences. It is known, however, that medial premotor areas, including the pre-SMA, seem less essential when a specific movement is to be selected on the basis of an association with a sensory cue (Chen et al. 1995; Deiber et al. 1996, 1999; Hadland et al. 2001; Shima and Tanji 1998a; Thaler et al. 1995). A pharmacological inactivation study of the pre-SMA emphasized the importance of two
factors, sensory-cued versus memory-guided sequence retrieval, in determining behavioral deficits (Shima and Tanji 1998a). Monkeys performed the same sequence for 11 trials. They were not impaired when they performed the sequence with the aid of visual cues to guide sequence selection (trials 1–5) but committed errors on 69% of the first memory-guided trials (trial 6). The error rate decreased on subsequent memory-guided trials (trials 7–11), suggesting an essential role of the pre-SMA in the initial retrieval or organization of the memory guided sequence.

The cue-change paradigm used in experiment 4 was devised to cue subjects to retrieve whole sequences in the absence of a parallel simple sensorimotor association to a specific individual movement (Fig. 2). In the cue-change paradigm, the screen color instructed subjects to either perform the same sequence as on the previous trial or to switch to a different sequence. Neither screen color was associated with any particular, individual response; the first response could only be selected once subjects had retrieved the correct sequence of movements by reference to the sequence on the previous trial.

An RT analysis confirmed that the cue-change experiment was performed in a very different manner to the cue-sequence task. In the cue-sequence task (experiment 3, Figs. 8 and 9), there was no RT cost of switching from one sequence to the other, suggesting that it was no more difficult to select the first movement of a sequence even if that sequence had not been performed for several trials. This might be expected if the subject did not need to retrieve the whole sequence before selecting the first movement but if instead the subject was able to select the first movement on the basis of its association with the initiation cue. In the cue-change task (experiment 4, Figs. 10 and 11), there was a significant cost of switching to a different sequence. In the cue-change experiment, it was more difficult to retrieve the first movement if that sequence had not been performed recently. This would be expected if the first movements were only selected after the sequence had been retrieved by reference to the memory of the sequence performed on the previous trial. Now when TMS was applied over the pre-SMA, the first movement of the sequence was consistently and significantly disrupted (Fig. 10A). The application of TMS over the PMd did not significantly disrupt performance (Fig. 10B).

In summary, the results suggest that the pre-SMA is critical for the initiation of a sequence chunk, whether that chunk occurs halfway through the sequence or even when it occurs at its very beginning. The only caveat is that for the deficit to be expressed, there must be no alternative route, such as a simple association between a sensory cue and an individual movement, by which the first movement in a chunk can be selected.

It should be noted, however, that in the cue-sequence paradigm in experiment 3, pre-SMA TMS was associated with a disruption of the later movements of the sequence. The association between the cue and the first movement might have preserved the first movement against the disruptive effect of pre-SMA TMS in experiment 3, but the delay of the later movements suggested that TMS of the pre-SMA still affected the planning of the subsequent movements of the sequence. Thus in both experiments 3 and 4, pre-SMA TMS disrupted the total time to perform the sequences (Figs. 9A and 11A). TMS over PMd never disrupted the first movement RTs or the total sequence times in experiments 3 and 4 (Figs. 8–11B).

The effect of the pre-SMA TMS on total sequence time attests to its disruptive effect on the selection of superordinate sets of movements rather than just individual movements. Gerloff et al. (1997) have previously reported that TMS in an adjacent medial frontal location disrupted future movements in a metronome paced sequence. Gerloff and colleagues emphasized the increase in errors on subsequent movements when TMS was applied over a slightly more posterior position than the one investigated in the present study. The RT effects and the absence of errors after pre-SMA TMS in the present study may be a consequence of the lower stimulation frequencies used. Another possibility, however, is that the high error rates reported by Gerloff et al. was a result of the TMS’ greater proximity to the SMA region which might be more closely concerned with the execution of individual movements (Lee et al. 1999; Matsuzaka et al. 1992).

Specificity of TMS application over the pre-SMA

The design of the current set of experiments was intended to test the specificity of the pre-SMA, within the wider premotor region, for sequence organization. Although PMd TMS has been shown to affect movement selection in other situations (Johansen-Berg et al. 2002; Praamstra et al. 1999; Rushworth et al. 2002; Schluter et al. 1998, 1999), it did not affect the organization of movement sequences in the present investigation. Between-group analyses confirmed that the disruptive effects of TMS applied at the chunk point in experiment 2 and at the initiation of a sequence in experiment 4 were anatomically specific to the pre-SMA. Applying TMS over the PMd region also controls for any disruptive effects of the tactile and auditory sensations that accompany the TMS pulses; the PMd results from experiments 2 to 4 demonstrate that such sensations were not responsible for sequence disruption when TMS was applied over the pre-SMA.

Although the pre-SMA TMS was biased slightly away from the midline and toward the left hemisphere, it is still likely the pre-SMA region in both hemispheres was affected by the TMS. It is possible that the TMS over the midline had a larger disruptive effect than PMd TMS because it affected both hemispheres. Previous experiments have suggested that the effects of lateral hemisphere TMS are not just less pronounced versions of the effects of midline TMS; TMS over the midline in the pre-SMA or SMA region has been shown to produce distinct effects compared with stimulation over the more lateral hemisphere in the region of PMd or M1 (Gerloff et al. 1997; Schluter et al. 2002). Moreover the left PMd was studied in the present experiments because previous experiments have suggested that the PMd in the left hemisphere has the dominant role and exerts a bilateral influence over both hands (Johansen-Berg et al. 2002; Schluter et al. 1998). An asymmetry in the strength of transcortical inhibition in the motor system means that left hemisphere TMS has a greater effect on the cortex of the right hemisphere than vice versa (Netz et al. 1995). Despite these considerations, it remains possible that the administration of bilateral PMd TMS, or TMS over some other combination of cortical areas, might have a more disruptive effect on movement sequences. However, muscimol inactivation of the pre-SMA in just one hemisphere is sufficient to cause bilateral movement sequencing deficits (Nakamura et al. 1999), sup-
porting the view that unilateral disruption of either the PMd or the pre-SMA can have bilateral movement interference effects.

Bimanual sequences were chosen in the current set of experiments to minimize relational patterns (runs, repetitions, transpositions, etc.) that might influence subjects to organize a sequence in a specific way. It has recently been shown that medial frontal TMS can disrupt various aspects of bimanual coordination (Obhi et al. 2002; Serrien et al. 2002; Steyvers et al. 2003), suggesting that the disruptive effects of pre-SMA TMS in our study may be attributed to the use of bimanual sequences rather than the demands of organizing sequences. However, there are several lines of evidence that suggest the medial frontal region isn’t limited to bimanual tasks but that this region may have a more abstract role in the organization and performance of complex actions (Picard and Strick 1996). The disruptive effects of medial frontal TMS in the aforementioned coordination studies has been in the form of deterioration in the timing between the hands and therefore may underlie a role of the medial frontal cortex in temporal aspects of actions. Indeed, it has previously been suggested that the medial frontal cortex is involved in the temporal organization of movements, even when they are unimanual in nature (Clower and Alexander 1998; Shima and Tanji 1998a). Second, TMS over the SMA has induced sequencing errors even on unimanual sequences (Gerloff et al. 1997), but this disruption was only evident when subjects performed highly complex sequences. Finally, it is not even necessary for subjects to make any movements for the pre-SMA BOLD signal to increase when encoding interval sequences (Schubotz and von Cramon 2001) or switching response set (Brass and von Cramon 2002). That it should be the organizational demands of a movement task rather than simpler movement selection or execution demands that determine pre-SMA recruitment is consistent with the area’s closer connections with the prefrontal cortex than with the primary or spinal motor system (Bates and Goldman-Rakic 1993; Lu et al. 1994; Luppino et al. 1993).

The present series of studies did not address the role of the SMA in sequence organization. There are qualitative similarities in the activity patterns of SMA and pre-SMA neurons during sequence performance (Nakamura et al. 1998; Shima et al. 1996; Shima and Tanji 2000), and it is possible that TMS directed over the SMA may have had similar effects. Quantitatively, however, more pre-SMA than SMA neurons are activated in the updating of movements and movement sequences (Matsuzaka and Tanji 1996; Rushworth et al. 2002; Shima et al. 1996). Because TMS over more posterior midline regions such as the SMA has distinct behavioral effects to pre-SMA TMS (Steyvers et al. 2003), it may be possible to investigate the contrasting roles of the SMA and pre-SMA in sequence organization in future experiments.

Conclusions

The pre-SMA may have a general role in initiating a new set of responses whether the set is a chunk from a sequence as in the present study or in some single cell recording studies (Nakamura et al. 1998; Shima et al. 1996) or a set of response mappings (Brass and von Cramon 2002; Rushworth et al. 2002). As the sequence becomes more automated, by repeated performance, pre-SMA neurons become less active (Nakamura et al. 1998; Sakai et al. 1998; Shima et al. 1996). Another medial frontal region, the cingulate cortex, may have a role in changing responses with changing reinforcement contingencies (Hadayd et al. 2003; Rushworth et al. 2003; Shima and Tanji 1998b; Walton et al. 2002).

The pre-SMA does not have a general role in selecting or executing all movements, rather it seems to be concerned with the organization or selection of superordinate sets of movements. Pre-SMA TMS disrupts very few movements, and those that are disrupted are not necessarily those with longer RTs. If it is necessary to update the sequence during performance and initiate a new sequence chunk, then once again the pre-SMA is important. Such a function might be part of a more general role for the area in updating motor programs (Matsuzaka and Tanji 1996; Rushworth et al. 2002; Shima et al. 1996). A pre-SMA role in changing between sequence chunks may underlie increases in BOLD signal with increasingly complex sequences (Boecker et al. 1998) or when subjects are unexpectedly required to change sequence (Jancke et al. 2000).


