Changing the structure of complex visuo-motor sequences selectively activates the fronto-parietal network

V.S. Chandrasekhar Pammi, K.P. Miyapuram, Ahmed, Kazuyuki Samejima, Raju S. Bapi, Kenji Doya

Abstract

Previous brain imaging studies investigating motor sequence complexity have mainly examined the effect of increasing the length of pre-learned sequences. The novel contribution of this research is that we varied the structure of complex visuo-motor sequences along two different dimensions using mnx paradigm. The complexity of sequences is increased from 12 movements (organized as a 2×6 task) to 24 movements (organized as 4×6 and 2×12 tasks). Behavioral results indicate that although the success rate attained was similar across the two complex tasks (2×12 and 4×6), a greater decrease in response times was observed for the 2×12 compared to the 4×6 condition at an intermediate learning stage. This decrease is possibly related to successful chunking across sets in the 2×12 task. In line with this, we observed a selective activation of the fronto-parietal network. Shifts of activation were observed from the ventral to dorsal prefrontal, lateral to medial premotor and inferior to superior parietal cortex from the early to intermediate learning stage concomitant with an increase in hyperset length. We suggest that these selective activations and shifts in activity during complex sequence learning are possibly related to chunking of motor sequences.

Introduction

Most of our day to day activities involve acquiring and performing complex sequences of actions to achieve a desired goal, from lacing shoes to driving an automobile (Sun and Giles, 2001). Behavioral and neural correlates of motor sequence learning have been extensively studied using various paradigms (see Rhodes et al., 2004; Halsband and Lange, 2006 for reviews). Earlier neuroimaging studies that investigated sequence complexity have primarily manipulated the sequence length (e.g. 4, 8, 12 and 16 elements in Sadato et al., 1996; 1, 4, 12 and 16 elements in Catalá et al., 1998, 1999 and 4 to 8 elements in Boecker et al., 1998, 2002). However, these earlier studies equated sequence complexity (as defined by the sequence length) with the total number of movements to be learned. The length of the sequence forms only one possible dimension of complexity. Other manipulations of sequence complexity contrast between repeated and heterogeneous sequence of finger movements (for example, 11111 vs. 12312 Harrington et al., 2000; Haaland et al., 2004). They found that when sequence length is controlled, RT increases independently with the number of different responses or the number of transitions (e.g. 12222 vs. 12111 vs. 12122 vs. 12121; 12333 vs. 12133 vs. 12131). In the aforementioned paradigms one element is presented at a given time. These studies, however, have not addressed the complexity of motor sequences when the amount of information to be processed at a given time is systematically varied.

Miller (1956) introduced the concept of capacity limits on the amount of information that can be processed in immediate memory. By organizing information into a series of chunks, we can stretch the information bottleneck (Miller, 1956). Behavioral studies show that motor sequences are hierarchically organized with chunks of subsequences separated by long time gaps and increased number of errors (Rosenbaum et al., 1983). Chunking refers to the process whereby the motor sequence is recoded as an efficient representation performed with specific patterns of timing. Each chunk acts as a single memory unit and thereby overcoming the limitations of working memory, which was previously proposed to be 7±2 items (Miller, 1956) and later argued to be around 4 (Cowan, 2001). In a recent
study, Bo and Seidler (2009) observed that the visuo-spatial working memory capacity predicted the number of chunks and the rate of learning that the subjects could perform.

In previous behavioral studies, chunks were externally specified, such as change in pattern of movements (repetition, inversion and transposition, Koch and Hoffmann, 2000) or a temporal delay in response–stimulus interval (Verwey and Dronkert, 1996; Verwey et al., 2009). Spontaneous reorganization of unstructured sequences into a number of motor chunks has also been investigated (Sakai et al., 2003; Verwey and Eikelboom, 2003; Verwey et al., 2010). Particularly, according to this behavioral research, motor chunks include only 3–5 key movement elements and longer sequences are typically chunked already (Bo and Seidler, 2009; Terrace, 2001; Verwey, 2003). According to Verwey (2003; Verwey and Eikelboom, 2003) individual differences concealed shorter chunks within longer chunks consistent with a hierarchical representation of motor sequences (Bapi et al., 2005; Rosenbaum et al., 1983). However, longer chunk-size has also been reported (Kennerley et al., 2004 with chunk-size of 7 movements). Sakai et al. (2003) reported chunk-size between 1 and 5 sets of 2 elements each i.e. a maximum of 10 movements constituted a chunk, while performing a 20 movement long-sequence structured as a 2 × 10 task. Thus, increased complexity would be related to increased number of chunks and the phenomenon of chunking is intricately linked to performance of complex motor sequences. Although chunking in motor sequence learning has been extensively studied behaviorally, it has gained less attention in neuroimaging studies (see also Bor et al., 2003; Graybiel, 1998; Kennerley et al., 2004; Verwey et al., 2002).

Earlier neuroimaging studies indicate the involvement of the fronto-parietal network along with the premotor cortex in complex motor sequence learning (see Honda and Shibasaki, 1998 for a review). With increasing sequence complexity, increased activation in premotor, precentral and posterior parietal areas was observed, with decreases in activation in inferior parietal, and superior and dorsal frontal areas (Boecker et al., 1998, 2002; Catalan et al., 1998; Sadato et al., 1996). Harrington et al. (2000) observed that cerebellum and the premotor cortices activated more in complex compared with simple sequences irrespective of the hand used. The increase in the number of superior parietal areas were positively correlated with the number of frontal areas (Boecker et al., 1998, 2002; Catalan et al., 1998; Sadato et al., 1996). Harrington et al., 2000; Honda and Shibasaki, 1998; Sadato et al., 1996).

The mnx sequence learning paradigm (Bapi et al., 2000; Hikosaka et al., 1995) allows us to increase the complexity of sequence in two dimensions (m, n) without changing the sequence length (i.e. total number of movements to be learned). In the mnx sequence learning paradigm (Bapi et al., 2000), visual stimuli consisting of m illuminated squares on a 3 × 3 grid are displayed on a computer monitor. Subjects learn to press m corresponding keys (called a set) successively on a keypad in response to the visual stimuli. The order of key presses has to be discovered by trial and error. The complete sequence consists of n such sets (called a hyperset). For example, the 2 × 6 task (Fig. 1a) consists of learning a sequence of 12 movements by incrementally learning 6 sets of 2 elements each. The sequence length (12) is simply obtained by the multiplication of set length (2) and hyperset length (6). In our study, the amount of information to be processed at a time is represented by a set. The complexity of sequences is increased from 12 movements (organized as the 2 × 6 task) to 24 movements (organized as 4 × 6 and 2 × 12 tasks). This design allows us to investigate the effect of complexity along two dimensions (set length and hyperset length) without varying the sequence length (Fig. 1b).

We hypothesize that frontal areas are more active during early learning stages in which participants engage in trial and error learning of the sequence, and the parietal areas would be more active after initial learning (Jenkins et al., 1994; Sakai et al., 1998). Additionally, our design allows us to investigate brain areas involved in the process of chunking motor sequences. Due to the limitations on immediate memory span (Cowan, 2001; Miller, 1956) and based on previous findings on typical chunk lengths (Bo and Seidler, 2009; Verwey, 2003), we hypothesized that a smaller set-size (such as in 2 × 6, 2 × 12 tasks) would enable spontaneous chunking across several sets (Sakai et al., 2003), while increasing the set-size (such as in the 4 × 6 task) will limit the chunk formation to single sets (Pammi et al., 2004). In this context, our study should reveal different neural activations corresponding to mnx tasks in which chunking across sets is facilitated compared to when chunking is limited to single sets. The two dimensions of complexity would also point to possible differences during chunk formation and execution (Verwey, 2001; Verwey et al., 2010).

Materials and methods

Subjects

Eighteen right-handed normal volunteers (15 males and 3 females with a mean of 23.65 years) participated in this study. Of these 17 subjects were considered for data analysis as one subject did not show learning in the 2 × 6 task. Subjects were paid for their participation and written informed consent was obtained. The ethics committee of the Brain Activity Imaging Center (BAIC), Advanced Telecommunications Research Institute International (ATR), Kyoto, Japan approved the experimental protocol.

Stimuli

Subjects lay supine in the scanner and visual stimuli were projected on a mirror in front of them. In the mnx sequence learning task (Bapi et al., 2000; Hikosaka et al., 1995), a set of m squares was illuminated simultaneously in white color on a 3 × 3 grid display against gray background (Fig. 1a). The grid measured 3.7 cm × 3.7 cm on the rear screen and the viewing angle was approximately 5′ × 5′. For each set, subjects learned by trial-and-error the correct order of successively pressing m corresponding keys on a 3 × 3 keypad placed near their right hand. The complete visuo-motor sequence of mnx key-presses was composed of n such sets (called a hyperset), which was incrementally acquired by trial and error. Subjects were
 instructed to use their index, middle and ring fingers for the three columns of the keypad — left, middle and right columns, respectively. If subjects were unable to complete a set within a specific time period (a maximum of 0.8 s per key-press) or if they pressed an incorrect key, it was considered an error and indicated by a screen flash and the sequence was reset to the beginning (hyperset). If the subject successfully completed the set of m keys, the next set was presented, and so on until all the n sets were completed (Fig. 1a). To encourage smooth and skilled performance, subjects were allowed to proceed to the next set as soon as they completed the previous set. A hyperset was generated randomly for each of the simple and complex sequence tasks separately and the same sequence was repeatedly practiced. To reduce the possibility of any explicit structure or pattern in the sequence, the hyperset was generated such that any repetition or transposition of sets did not occur. The generation of sequences, presentation of visual stimuli and recording of responses was carried out with custom-built software running on a Macintosh computer.

**Task procedures**

Three experimental settings were used with a block design using alternating sequence (2 × 12, 4 × 6 and 2 × 6) and baseline (follow) conditions (Fig. 1c). The experimental settings with two complex sequence learning tasks (2 × 12 and 4 × 6) alternating with follow were performed in a single scanning session and their order was counterbalanced across subjects. The experimental setting with the 2 × 6 task alternating with follow was performed in a single scanning session. The order of simple (2 × 6) and complex (2 × 12, 4 × 6) experimental settings were counterbalanced across subjects. In the follow condition, the subjects were asked to press one key at a time following randomly generated visual targets and thus there was no sequence learning involved. The follow condition served as a baseline for the fMRI activations as it consisted of both visual input and generation of motor output (i.e. key-press). Further, as only one element was presented at a time, there was also no trial and error learning involved. Participants were required to complete the key-press within 0.8 s after presentation of the visual stimuli in the follow condition. In the sequence learning condition, they continuously practiced one of the m × n sequence tasks. Each experimental setting consisted of four sessions, each of which began and terminated with a follow block alternating with sequence blocks (Fig. 1c). Each session had 7 follow and 6 sequence blocks, each lasting for 18 s and 36 s, respectively. We adapted the asymmetric durations of the sequence and follow blocks to maximize the time for learning sequences within the duration of the fMRI scanning. Each block consisted of several trials (see Fig. 1a for an example trial). A trial begins with the presentation of the first set of the sequence and continues until the end of the sequence. Upon successful completion of the sequence (completion of the hyperset), a new trial begins. However the trial was reset (indicated by a screen flash) to the beginning of the sequence whenever an incorrect response or a time-out (0.8 s per key-press) error occurred. The reset (screen flash) signal allowed the participants to explicitly learn the complete sequence in an incremental fashion. Incorrect responses occurred if participants pressed a key that was not lit up and also if they pressed the lit keys in the incorrect order. We do not distinguish between incorrect responses and time-out errors and use the success rate (reflecting accuracy) as an index of learning (see section on behavioral parameters and analysis). For programming purposes, the follow blocks were considered to consist of 1 × 12 sequences. 21 random sequences (1 × 12) were generated and one of them was randomly selected on every trial. For sequence blocks, one sequence was
generated randomly that was repeatedly practiced. Before proceeding to scanning experiments, subjects practiced sequence and follow tasks for two sessions to become familiar with task procedures and instructions. Different hypersets were used for practice and scanning sessions. Thus, the participants learned a new sequence during the scanning different from the practice session.

**Functional imaging**

Functional images were acquired in a 1.5 T whole-body scanner (Shimadzu-Markoni Magnex Eclipse). Each experimental setting consisted of four scanning sessions with 7 follow and 6 sequence blocks, each lasting 18 s and 36 s respectively (Fig. 1c). Each block began with the relevant instruction screen lasting for 6 s indicating whether the ensuing block corresponds to a sequence (S) or a follow (F) condition. A time series of 228 whole-brain scans with repetition time (TR) 6 s were obtained for each experimental setting. In each whole-brain scan, a set of 50 axial T2*-weighted gradient-echo planar images [repetition time (TR) 6000 ms, echo time (TE) 55 ms, flip angle (FA) 90°, matrix 64×64, field of view (FOV) 192×192 mm and slice thickness of 3 mm] covering the whole brain were collected parallel to the anterior commissure–posterior commissure (AC–PC) line. In addition, high-resolution T1-weighted sagittal anatomical brain images consisting of 191 slices (TR 12 ms, TE 4.5 ms, FA 20°, matrix 256×256, FOV 256×256 mm and slice thickness of 1 mm) were collected for each subject.

**Behavioral parameters and analysis**

Performance improvement of subjects was determined by two parameters, namely Success Rate (SR) and average key-press Response Time (RT). For the sequence conditions, subjects learned the mnx sequence (hyperset) incrementally by completing n number of sets (each consisting of m elements). Success Rate (SR) was computed as the ratio of the number of successful hypersets or part thereof completed to the total number of hypersets attempted (expressed as percentage). For example, in a 2×6 task, if the participant completed 2 sets successfully in a trial, then the SR is 2/6 for that trial. A sequence block would consist of several such trials (during which repeated learning occurs by trial and error) for the fixed duration of 36 s. The total number of trials in the block equals the number of hypersets attempted and the average SR for that block is computed. For the follow blocks, there was no sequence to be learned and subjects responded to visual stimuli presented one at a time. Therefore, the Success Rate in follow blocks was measured as the ratio of correctly pressed keys to the total number of required key-presses (equivalent to number of visual stimuli presented) and was expressed as percentage. For both the sequence and follow conditions, the average key-press RT was measured as the average time required to complete a key-press in a successfully completed set.

To account for individual differences in the learning, performance for each participant was classified into different learning stages. Sequence learning would progress from an initial, early stage in which the participants are slow and attentive to a more automatic late stage. For each sequence condition, we identified the early learning stage into sessions that involved trial and error learning processes. The first trial in which the participants completed the hyperset was identified (see Supplementary material). The imaging data analysis used a session-separable model. At this resolution, we classified the early learning stage as beginning from the first session to the nearest session in which the trial completing the first hyperset was identified. For the 2×6 task, all the 17 subjects considered for data analysis had completed the first hyperset within the first session. For the 2×12 task, 15 subjects completed the first hyperset within the first session. For the 4×6 task in which there was greater trial and error learning involved, we observed that only 7 subjects completed the first hyperset within the first session. For both the 2×12 and 4×6 tasks, the early learning stage defined by the criterion of first hyperset completion did not extend beyond the third session. The session-wise analysis (see Supplementary material) indicated that asymptotic learning was observed only in the 2×6 task by the fourth session. Further, the average number of sequences completed in the sequence tasks were as follows: 2×6: 126±6; 2×12: 35±3; 4×6: 32±4 (mean±standard error). Hence, the fourth session in the 2×6 task would correspond to the late stage—i.e. performance of over-learned sequences. We marked the intermediate learning stage as the 2nd and 3rd sessions for the 2×6 task. For 2×12 and 4×6 tasks, there was no late learning stage within the four sessions. Hence after marking the early learning stage, the remaining sessions in these two tasks were designated as belonging to the intermediate learning stage. The potential confound of not having the movements paced (in order to allow smooth execution of sequences) would result in different number of movements across conditions and across participants. We accounted for this by analyzing the number of finger movements between the three sequence tasks (see Supplementary material). The statistical analysis on number of movements indicated that the total number of movements performed across the complex sequence learning conditions (2×12 and 4×6) was similar.

Repeated Measures ANOVA was performed separately for the two measures—success rate (SR), the average key-press response times (RT) by entering two factors, namely, Tasks (3—2×6, 2×12, 4×6) and stages (2—Early, Intermediate). The analysis was carried out on the average behavioral measures calculated for early and intermediate stages. Since the 4th session data in the 2×6 task corresponded to the late learning stage, this data was not included in the analysis. The analysis was carried out separately for follow and sequence conditions.

**Statistical analysis of functional images**

The imaging data were analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, University College London) using MR option (Friston et al., 1994, 1995a, 1995b). Functional images were reoriented to align the origin near the intersection of the coronal plane through the Anterior Commissure and the line joining the AC with the PC (Posterior Commissure). Other preprocessing steps included realignment, normalization and smoothing with an 8 mm Gaussian kernel. Each individual subjects’ data were analyzed using a session-separable general linear model modeling the sequence and the control conditions using a box-car function convolved with canonical HRF. Contrast images comparing the sequence with follow condition were generated for each subject for each session. Group analysis followed the random effects approach using a within-subjects model grouping the session-wise contrasts into early and intermediate stages. The data from the late stage of 2×6 was included in the model but not analyzed. Linear contrasts comparing the complex sequence conditions (2×12, 4×6) with the simple sequence condition (2×6) would not distinguish brain activation specific to the dimension of complexity from the increased sequence length. Hence, we setup orthogonal contrasts to test for dimensional changes in complexity while accounting for the increase in sequence length. The contrast for set-increase effect tested for greater activation in 4×6 compared to 2×6 and 2×12 tasks (contrast weights [−1 −1 2] for [2×6 2×12 4×6]), and the contrast for hyperset-increase effect tested for greater activation in 2×12 compared to 2×6 and 4×6 tasks (contrast weights [−1 −2 −1] for [2×6 2×12 4×6]). General sequence learning-related decrease and increase in activity across learning stages was assessed using linear contrasts (contrast weight of 1 and −1 for early and intermediate stages and vice versa). All activations are reported at an exploratory threshold of p<0.001, uncorrected for the whole brain. We simultaneously indicate whether the activations survived stringent thresholds corrected for multiple comparisons i.e. controlled for false discovery rate (FDR, corrected for
activated voxels only) and family wise error (FWE, whole-brain) correction.

**Results**

**Behavioral results**

Subject specific improvements in learning were assessed by a simple ANOVA for each subject and for each experimental setting comparing the six sequence blocks of session 1 and session 4. One of the eighteen subjects did not show significant improvements on the 2×6 task [RT: F(1,10) = 0.14, p = 0.71 and SR: F(1,10) = 2.42, p = 0.14] and hence was excluded from further analysis.

Repeated Measures (RM) ANOVA performed on the two behavioral parameters for the control (follow) condition yielded no significant difference across the three experimental settings [SR: F(2,32) = 0.64, p = 0.5318; RT: F(2,32) = 0.932, p = 0.404], thereby indicating a steady performance in the follow condition across the experimental settings.

The average behavioral parameters (SR, RT) were computed for the early and intermediate stages in each sequence condition. Learning was observed in each of the three sequence conditions – 2×6, 2×12 and 4×6 from early to intermediate stages with significant increases in Success Rate (SR) and corresponding decreases in Response Times (RT) (all p<0.001). For both learning stages (early and intermediate) there was significant difference between the simple (2×6) and complex (2×12, 4×6) conditions (SR and RT: p<0.0001). Interestingly when we compared the two complex conditions – 2×12 and 4×6, the Success Rates (SR) were at similar levels [Early: F(1,32) = 0.435, p = 0.51; Intermediate: F(1,32) = 0.01, p = 0.92] but the Response Times (RT) showed a differential behavior. RTs were similar [F(1,32) = 0.232, p = 0.633] in the Early period. On the other hand, in the intermediate stage, RTs were significantly [F(1,32) = 6.99, p = 0.012] faster in the 2×12 compared to the 4×6 task (Figs. 2a and b).

We probed the reason for smaller RT in the 2×12 task compared to the 4×6 task by analyzing the chunking patterns as revealed by the temporal profiles of set completion times of individual sets. Based on earlier studies, we mark the beginning of a chunk by identifying significant pauses in the temporal profile (Koch and Hoffmann, 2000; Rosenbaum et al., 1983; Sakai et al., 2003; Terrace, 2001; Verwey and Dronkert, 1996). A simple one factor ANOVA (different set completion times were treated as the between group and different subjects were treated as within group sources of variance) revealed significant differences in set completion times for 2×6 (F(5,96) = 7.53, p<0.001) and 2×12 tasks (F(11,192) = 2.32, p<0.05) but not for the 4×6 task (F(5,96) = 0.22, p>0.95). Fig. 2c shows the average set completion times for different sets for the three sequence conditions. The set completion times for the 2×6 task showed considerably greater RTs for completion of the first set compared to sets 2 to 6 (Fig. 2c). This suggests that there was a pause at the beginning of the hyperset and that the entire hyperset could be considered as one motor chunk. For the 4×6 task, all sets required similar amounts of time to complete, suggesting that each set was a distinct motor chunk. For the 2×12 task, a modulation of set completion times was observed and certain sets took a longer time to complete compared to other sets, suggesting that participants spontaneously organized the sequence into a number of motor chunks (Fig. 2c). These results demonstrate that there was a pause at the beginning of the hyperset and chunking across sets does occur in the 2×12 and 2×6 tasks but not in the 4×6 task. In the 4×6 task, chunking perhaps occurs only within sets, but this cannot be confirmed as we did not record the individual keypress times in this study. While the results depicted in Fig. 2c demonstrate the average chunking patterns across subjects, the actual chunking patterns are different for each subject.

The behavioral results suggest that there were differences in response times between the two complex conditions – 2×12 and 2×12 and 4×6, which could possibly be explained by different chunking strategies employed for these two tasks.

**Functional imaging results**

Contrasts of interest in the present study looked at activations related to the two dimensions of complexity of movement sequences – set length increase effects and hyperset length increase effects. In addition, we probed these contrasts in early and intermediate stages of learning.

Brain activations related to hyperset length increase effect were assessed by contrasts that implement larger weights for the 2×12
task compared to 2×6 and 4×6 tasks (Fig. 3) separately for early (Table 1) and intermediate (Table 2) stages. In the early stage of hyperset increase effect, strongest activation was found in the inferior parietal and lateral prefrontal regions (Table 1). A remarkable increase in the extent of parietal activation from the early to intermediate stage was observed (Fig. 3). Early and Intermediate activations shown in blue and red, respectively. While unilaterial inferior and superior parietal activation was observed in the early stage (Table 1, Fig. 3), the activation in the intermediate stage expanded into a large cluster spanning medial, inferior and superior parietal areas bilaterally (Table 2, Fig. 3). The bilateral activation in the lateral prefrontal areas in the early stage (Table 1) was more prominent in the left hemisphere in the intermediate stage (Table 2). There appears to be a shift in activation from the ventral to dorsal prefrontal cortex across learning stages. There was an increase in the extent of the premotor activation from the early (Table 1) to intermediate stage (Table 2) and the premotor activation was more dorsal in the intermediate stage (Fig. 3). Activation in the pre-supplementary motor area (Pre-SMA) was also observed in the intermediate stage (Table 2, Fig. 3). These activations suggest a role for the fronto-parietal network in relation to the increased hyperset length of visuo-motor sequences.

Other brain areas that were activated in response to increases in the length of the hyperset were the medial temporal lobe and hippocampus in the early stage (Table 1) and the inferior/middle occipital areas and striatum in the intermediate stage (Table 2). We also note the transition of activation from the posterior cingulate cortex (Table 1) to anterior cingulate cortex (Table 2) from early to intermediate stage, respectively.

Brain activation related to the increase in the length of the set was assessed by constructing contrast with larger weight corresponding to the 4×6 task compared to 2×6 and 2×12 tasks, separately for early and intermediate stages. Surprisingly, we found little activation due to set length increase effects and hence included a separate table in the Supplementary material (Table A3). The right superior occipital lobe (BA 18/19, MNI coordinates 27, −66, 18; T value, 4.14; no. of voxels, 20) was activated in the early learning stage and the middle cingulate cortex (BA 21/31, MNI coordinates, −15, −21, 33; T value 3.92; no. of voxels, 14) was activated in the intermediate stage.

The general sequence learning-related decrease (Table 3) and increase (Table 4) in brain activations were assessed using the contrasts that compared the early and intermediate learning stages (Fig. 4; blue map for decreasing and red map for increasing activations) irrespective of the type of sequence condition (2×12 or 2×6).
from early to intermediate stages are the left putamen (z = −6), SMA (z = 44, 54), and left M1 (z = 54). Activations increasing as indicated by cross-lines on the medial sagittal plane. Activations decreasing from early to intermediate stages shown in the generic network associated with the trial-and-error sequence learning (including primary motor area M1). These brain regions represent a putamen, the supplementary motor area and the left precentral gyrus and ventral) regions, the right dorsal premotor cortex showed activations. While the activation decreased in the left premotor (dorsal) areas (common to 2×12, 4×6, and 2×6 tasks) increasing from early to intermediate stage.

### Table 3
General sequence learning areas with decreasing activations across learning stages.

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Coordinates (mm)</th>
<th>T score (number of voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle occipital cortex L</td>
<td>39/19</td>
<td>36 − 69 18 4.66 (26)*</td>
</tr>
<tr>
<td>Inferior parietal cortex R</td>
<td>39/40</td>
<td>42 − 42 33 4.54 (112)*</td>
</tr>
<tr>
<td>Anterior insula</td>
<td>40/39</td>
<td>− 45 − 54 42 4.00 (56)</td>
</tr>
<tr>
<td>Premotor (dorsal)</td>
<td>47</td>
<td>− 30 21 − 6 4.69 (10)</td>
</tr>
<tr>
<td>Premotor (ventral)</td>
<td>6</td>
<td>− 36 20 30 3.61 (10)</td>
</tr>
<tr>
<td>Ventrolateral prefrontal cortex</td>
<td>44</td>
<td>51 21 33 5.86 (223)**</td>
</tr>
<tr>
<td>Superior frontal cortex R</td>
<td>45/44</td>
<td>− 45 21 27 4.93 (146)**</td>
</tr>
<tr>
<td>Lateral orbitofrontal cortex</td>
<td>4/10/11/47</td>
<td>30 57 6 5.27 (74)**</td>
</tr>
<tr>
<td>Precentral gyrus/primary motor area</td>
<td>47/46</td>
<td>− 48 45 3 4.42 (90)*</td>
</tr>
<tr>
<td>Medial frontal cortex R</td>
<td>8/9</td>
<td>33 24 57 4.99 (102)**</td>
</tr>
<tr>
<td></td>
<td>32/8</td>
<td>6 30 45 4.79 (74)*</td>
</tr>
</tbody>
</table>

* Indicates FDR corrected activations with p < 0.05, number of voxels ≥ 10.
** Indicates FWE corrected activations with p < 0.05.

### Table 4
General sequence learning areas with increasing activations across learning stages.

<table>
<thead>
<tr>
<th>Brain area</th>
<th>BA Coordinates (mm)</th>
<th>T score (number of voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putamen L</td>
<td>6/4</td>
<td>− 27 21 60 6.25 (285)**</td>
</tr>
<tr>
<td>Premotor (dorsal)</td>
<td>6</td>
<td>24 − 6 60 3.65 (14)</td>
</tr>
<tr>
<td>Supplementary motor area (SMA)</td>
<td>0</td>
<td>0 48 4.21 (74)*</td>
</tr>
<tr>
<td>Precentral gyrus/primary motor area</td>
<td>6/4</td>
<td>− 27 21 60 6.25 (285)**</td>
</tr>
</tbody>
</table>

* Indicates FDR corrected activations with p < 0.05, number of voxels ≥ 10.
** Indicates FWE corrected activations with p < 0.05.

Learning of visuo-motor sequences is facilitated by chunking

Behavioral results indicated differential performance across the two complex conditions – 2×12 and 4×6. Increases in the set-length (increase of m in 2×6 versus 4×6 tasks) would increase the short-range prediction load while acquiring the sets. Similarly, increasing the hyperset-length (increase of n in 2×6 versus 2×12 tasks) would be related to the increase in long-range prediction load (acquisition of long hyperset). The decreased RTs in the 2×12 task but not the 4×6 task could possibly be attributed to the differences observed in sequence organization into a number of motor chunks (Bapi et al., 2005; Miyapuram et al., 2006; Pammi et al., 2004; Sakai et al., 2003; Verwey and Eikelboom, 2003; Verwey et al., 2010).

A classical signature of a chunk in the context of movement sequences is that some elementary movements have longer response times than others resulting in a zig-zag temporal profile (Rosenbaum et al., 1993; Terrace, 2001). Using an mxn task, Sakai et al. (2003) found that participants developed a characteristic temporal profile across sets (chunking). When the sequence was reordered by shuffling the sets, performance was poorer when the chunk patterns were disrupted compared to when they were preserved. We extend the previous research (Sakai et al., 2003; Verwey, 2003) by demonstrating that the set-size does influence the formation of...
chunks possibly due to the limited capacity of immediate memory (Bo and Seidler, 2009).

We observed that possibility of spontaneous chunking of movements across sets is more likely when the set-size is small, as in 2×12 and 2×6 tasks. In contrast, in the 4×6 task where the short-range load (set size) is increased, motor chunk formation did not seem to extend across sets (see Fig. 2c). An alternative explanation is that subjects might be chunking only the movements within the set. This is likely in the 4×6 task, but cannot be confirmed from our data as we did not record individual key-press times. Recent behavioral findings from our group (Singh, 2010) confirm that within-set chunking is the dominant pattern in the 4×6 task.

In the current experiment, by arranging the same number of movements (24) in two different ways (4×6, 2×12), while the amount of learning as indicated by success rate is similar in both cases (Fig. 2b), an efficient execution of the sequence as indexed by faster response times (Fig. 2a) is facilitated by chunking across sets.

In possible extensions of the current study, it would be interesting to investigate the effect of manipulation of visuo-spatial working memory times (Fig. 2a) is facilitated by chunking across sets.

Based on the current study, we can speculate that low visuo-spatial working memory load facilitates chunk formation of movement sequences across sets.

Selective involvement of fronto-parietal cortices with an increase in long-range complexity

A preferential activation of fronto-parietal network was observed with an increase in hyperset length (2×12 task). Sakai et al. (1998) found that during visuo-motor sequence learning, there was a transition of brain activation from frontal areas to parietal areas. Other studies on sequence complexity (Boecker et al., 2002; Catalan et al., 1998, 1999; Harrington et al., 2000; Janata and Grafton, 2003; Sadato et al., 1996) found increased parietal activation in response to increasing the sequence length. Our results support these previous studies of parietal involvement to increased complexity of visuo-motor sequences. Further we extend the previous research by demonstrating that the parietal involvement is selective to increasing the hyperset length (from 6 sets in the 2×6 task to 12 sets in the 2×12 task). Further, this effect cannot be explained solely due to increasing the total number of movements required to complete the sequence (from 12 movements in the 2×6 task to 24 movements in 2×12 and 4×6 tasks), as the 4×6 task also has increased number of movements but no selective activation observed. However, we do not know whether selective activation of parietal cortex to increased hyperset length is restricted to sets of length 2 or whether it is also true for sets of length >2. We conclude that the selective activation of parietal cortex is related to processes involved in dealing with long-range complexity.

Differential activation supporting early and intermediate stages of complex sequence learning

Activation in the parietal cortex shifted from the inferior parietal areas in the early stage to the posterior/superior parietal cortex in the intermediate stage of the 2×12 task. The inferior parietal cortex is said to be involved in building (encoding) an abstract representation of complex sequences and the superior parietal cortex is known to translate a plan into an action/goal (Harrington et al., 2000). In our study, the inferior parietal cortex may have a similar function in the abstract representation of 2×12-like tasks. This may be related to the process of learning to string together consecutive sets in order to efficiently learn long-range sequences. Bapi et al. (2006) suggested that sequence learning in early stage is supported by an abstract representation in the parietal cortex that enables a quick acquisition of the sequence and an effector-specific representation in the premotor cortex enabling efficient performance in the late stage. Our behavioral data support the formation of motor chunks that facilitate efficient performance in the intermediate stage. It appears that while inferior parietal cortex builds representations, the superior parietal cortex helps in retrieving them. For example, the inferior parietal cortex might be involved in encoding a chunk representation for a mobile phone number such as 9440746382 into three chunks, say, 94407, 463 and 82. The superior parietal cortex would be involved in predicting what comes next after 94407 and so on. The premotor cortex would then be involved in representing an effector-specific motor program for each chunk.

Activity in the prefrontal cortex in the 2×12 task shifted from ventral to dorsal areas from early to intermediate stage, respectively. The ventrolateral prefrontal cortex (VLPFC) has been suggested to play an important role in the maintenance of the order of sequence of movements (Doyon et al., 2002). Badre and D’Esposito (2009) suggested that the ventral frontal regions process the context during retrieval or selection of relevant information. Thus it is possible that the ventral frontal region activated in the early stage of the 2×12 task indicates its involvement in acquisition of relevant information required during sequence learning. Amiez and Petrides (2007) suggested a role for dorsolateral prefrontal cortex (DLPFC, BA 46 or 9/46) in maintaining the order of short sequences whereby this representation would be translated into motor actions with the help of motor related areas. Consistent with this, the activity in DLPCF observed during the intermediate stage of the 2×12 task may be related to the online maintenance of motor programs (chunks) to enable execution.

These results suggest that activation in fronto-parietal network shifts from ventral (inferior) and lateral axes towards more dorsal (superior) and medial areas during complex motor sequence learning. Further, we speculate that the functional role of these areas is to support aspects of chunk formation and execution.

Cortico-subcortical networks supporting learning and performance of complex visuo-motor sequences

The basal ganglia circuitry was suggested to be involved in the formation of rules (Bébard and Sanes, 2009) and the activation in the intermediate stage of long-range sequences (2×12 task) would possibly imply its role in forming rules required for long-range sequence performance (i.e., already learned sequence). It was also suggested that the basal ganglia are involved in the process of motor chunking (Boyd et al., 2009; Graybiel, 1998) and the activity in our study in the intermediate stage may suggest its role in the formation of hierarchical representations of learned long-range sequences through the process of chunking.

The pre-SMA activity was implicated in the acquisition of new sequences (Hikosaka et al., 1996) whereas Sakai et al. (1998) observed its activity in the early and intermediate stages. The activity in the intermediate stage of our study seems to have a similar interpretation as in Sakai et al. (1998). It is possible that pre-SMA plays an active role along with the dorsolateral prefrontal cortex (DLPFC) and the dorsal premotor area during intermediate stage to form a motor sequence representation leading to the formation of motor programs for long sequences. It is possible that these secondary motor and memory related areas might be playing a critical role in facilitating motor chunking processes. Indeed the pre-SMA has been proposed to be a candidate brain region in updating chunks (Kennerley et al., 2004; Nakamura et al., 1998; Sakai et al., 2003). Based on the earlier studies, Verwey (2001) proposed that the dorsol prefrontal cortex and anterior cingulate may have a role as cognitive processor and that the motor processor comprises the supplementary motor area, the basal ganglia and the lateral cerebellum. The general finding that the (left) putamen, supplementary motor area (SMA), and left primary motor area (M1) showed increasing activity (Table 4)
is in line with the model of Verwey et al. (2002). It states that with practice the basal ganglia increasingly trigger the SMA (to concatenate motor chunks), and that the SMA in turn triggers M1 (to execute the elements of a motor chunk).

Activity in the premotor area has been consistently reported during the execution of sequential finger movements of increasing complexity (Boecker et al., 2002; Catalan et al., 1998, 1999; Harrington et al., 2000; Janata and Grafton, 2003; Sado et al., 1996). It is possible that it might be involved in the storing of sequence plans thereby acting like a motor working memory. We could not observe differential activity in the cerebellum even though our imaging procedure allowed us to scan this region. We believe this is due to our subjects learning sequences with the help of a trial-and-error process, an example of reinforcement learning (Doya, 2000) for which basal ganglia are implicated. The role of the cerebellum needs to be established in future studies investigating supervised and reinforcement learning modes of sequence learning.

The fact that we observe activation in the cortico-striatal pathways (Alexander et al., 1986) adds to the traditional view of their role underlying the chunking of motor sequences such as formation, reinforcement learning modes of sequence learning.


