Motor timing and motor sequencing contribute differently to the preparation for voluntary movement

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A B S T R A C T

Two crucial processes preceding voluntary action are determining the time for movement initiation and planning of the specific sequence of motor output. In this study we aimed to differentiate the neural activity related to motor timing and motor sequencing and to examine over what time periods they contribute to premovement activity during the readiness for voluntary action. Eighteen participants performed self-initiated voluntary finger movements in a readiness potential paradigm, both during EEG measurement and during fMRI. The finger movement task involved three conditions: (1) simple repetitive sequences; (2) increased demand on the sequencing of movement order; and (3) increased demand on the timing of movement initiation. Functional MRI and 64 channels EEG were conducted in two separate sessions. Motor timing and motor sequencing were found to involve different neural processes occurring at different times prior to movement initiation. Motor timing involved greater activation in lateral prefrontal regions over the earliest part of premovement activity, from 1200 ms before movement onset. Motor sequencing involved greater activation of dorsal premotor and parietal areas and was reflected in central and parietal scalp regions only over the later part of premovement activity, within 600 ms of movement onset. We suggest that different neural processes contribute to different aspects of the intended action over different time periods during the preparation for movement, and it is the coordinated activity of these multiple regions that is represented in premovement activity during the readiness for voluntary action.

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Introduction

The ability to plan and perform voluntary action is essential to express our intentions and manipulate the environment in relation to our own will. Every voluntary movement is preceded by brain activity aimed at preparing and executing the action. When movements are self-initiated, i.e. performed at our own will without any external cue, this brain activity can start up to 2 s before the execution of the movement. The activity appears in the EEG as a slow-rising negative potential that has been called Bereitschaftspotential or Readiness Potential (RP) (Deecke, 1969; Kornhuber and Deecke, 1965). Neuroimaging studies have shown involvement of a widespread higher-motor network in the preparation for action, including the supplementary motor area (SMA), premotor cortex, basal ganglia, inferior and superior parietal lobes, and prefrontal regions including the anterior cingulate (Ball et al., 1999; Cunnington et al., 2002, 2003; Deiber et al., 1999).

Premovement activity is suggested to involve two major components: an abstract level of movement preparation and intention to move followed by specific programming for movement execution. The former component is reflected in the early readiness potential and premovement activity of regions including pre-SMA, prefrontal cortex, lateral premotor areas and parietal lobe. The latter component has been associated with the late readiness potential and activity of the primary motor cortex (M1) and SMA proper (Shibasaki and Hallett, 2006).

The function of the mesial motor areas (pre-SMA, SMA-proper and cingulate motor area) during preparation for movement and the specific cognitive or motor processes that contribute to the earliest component of premovement activity are unclear. Two crucial processes preceding voluntary action are determining when to initiate the action and determining the order of movements that are involved in the action. The former process we refer to in this study as motor timing and corresponds to the internal decision on when to perform a voluntary action (Deecke, 1996). It must be noted that the motor timing we examine here is the process of determining the appropriate time for movement initiation. This may be distinct from other forms of motor timing involved in rhythmic movement or coordinating the timing of sub-movements within a sequence (Bengtsson et al., 2005). The later process, motor sequencing, involves the planning of the specific sequence of motor output required to achieve the intended goal of the action. In this study, we examine the contribution of processes related to the timing of movement initiation and motor sequencing to premovement activity during the preparation and readiness for voluntary action.
Previous studies have shown a role of the SMA in both the timing and the sequencing of movement. Neuroimaging studies show that there is a positive correlation between SMA activity and the ordinal complexity of a sequence of movements (Boecker et al., 1998; Sadato et al., 1996a). Moreover intracranial recordings in monkeys have shown that SMA activity is partly related to the selection of a specific sequence order (Shima and Tanji, 1998, 2000). Neuro-cognitive models of time estimation have pointed to the SMA and fronto-parietal striatal circuits as the neuronal substrate of an internal clock that creates representation of time (Macar et al., 2004, 1999; Meck and Benson, 2002) on which mechanisms of movement initiation rely. Studies on motor timing show that premovement activity in the SMA is affected by rhythm complexity (Bengtsson et al., 2005; Chen et al., 2008; Dhamala et al., 2003; Lewis et al., 2004) and when maintaining movement rhythm in the absence of external cues (Rao et al., 1997).

Motor timing and motor sequencing, however, are also separable processes, as previous studies show that some brain regions have a preferential role in motor timing while others are more involved in motor sequencing.

Numerous studies highlight the crucial role of a fronto-parietal circuit in movement sequencing (Bengtsson et al., 2004; Catalan et al., 1998; Rushworth et al., 2001a, b, 1997; c; Sadato et al., 1996b). Rushworth et al. (2001b) studied the effects of motor attention to movement sequencing, showing enhanced activity in the cingulate motor area, dorsal lateral premotor area and intraparietal sulcus when participants specifically attended to sequencing movements. Moreover, sequence preparation is disrupted by stimulation of the parietal cortex by transcranial magnetic stimulation (Rushworth et al., 2001a) and patients with parietal damage show deficits in using advance information for movement sequencing (Rushworth et al., 1997). Bengtsson and co-workers suggested that the posterior parietal area may process trajectories of movements, while the lateral frontal area and the inferior parietal area may be involved in creating abstract representation of sequences of elements (Bengtsson et al., 2004). Therefore ordering movements in sequence seems to rely on a neural circuit involving frontal and parietal areas.

Other studies show that attention to motor timing and decision on when to move specifically involve activity of the right dorsolateral prefrontal cortex (DLPFC) (Lewis and Miall, 2003, 2006). In a recent time processing model (Lewis and Miall, 2003), SMA and DLPFC have been referred to as key structures for time processing. In this model both these areas are involved in time management but they play different roles. During automatic time processing, SMA may act as an internal clock to create a representation of time intervals. Under cognitively controlled time processing an auxiliary internal clock may also be activated in the right prefrontal cortex. This model is in line with evidence of DLPFC involvement in non-routine decision making on the timing of movements (Jahanshahi and Frith, 1998; Jahanshahi et al., 1995; Jenkins et al., 2000).

In this study, we compared in the same paradigm the process of ordering movements in a sequence with the process of timing for movement initiation and the decision on “when to move.” We aimed to differentiate the pattern of neural activity related to each process and to examine when these different processes contribute to neural activity prior to movement initiation. We employed a self-paced movement task in which we separately manipulated motor timing and motor sequencing. We compared a condition of simple repetitive sequences with two conditions of high processing demand related to movement timing and sequencing respectively. In one, we increased demand on movement sequencing by alternating trial-by-trial between two complex finger sequences. In the other, demand on motor timing was increased by alternating trial-by-trial between two different time intervals between sequences. We used ERPs to identify the critical time periods during movement preparation for processes related to the timing of movement initiation and those related to sequencing of movement order. We also used fMRI to identify the brain areas involved in these two processes. In this way we were able to investigate both when and where motor timing and motor sequencing contribute to the preparation for voluntary action.

Method

Participants

Eighteen young healthy volunteers (7 females and 11 males; mean age: 25.5±2.85 years) participated in the experiment and gave their informed consent. All subjects were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). Data of one participant was excluded from EEG analyses due to technical problems during the EEG recording.

Task

Participants were asked to perform fast self-paced sequences of six consecutive movements and to interpose intervals of several seconds between sequences (Fig. 1). The sequences were executed with four fingers of the right hand (2–index, 3–middle, 4–ring, 5–small finger) by pressing four keys, one for each finger, on a response keypad.

As shown in Fig. 1, the specific sequences to perform and the timing or temporal range required for the initiation of each sequence were manipulated across conditions in order to independently vary task difficulty for movement sequencing and timing. There were three conditions.

Simple (Si): The sequence to be performed involved only adjacent finger movements (2–3–4–5–4–3) in a scale sequence. Participants were required to initiate movements within a relatively large time range, from 5 to 12 s after the end of the previous sequence. Therefore the task was relatively simple and repetitive and without strict timing demand.

Timing (Ti): Participants performed simple scale sequences (2–3–4–5–4–3); however, they were required to initiate each sequence within two narrow time ranges alternating between trials, from 3 to 6 s and from 7 to 10 s after the end of the previous sequence. This required participants to attend closely to movement timing, to initiate movements within the correct time ranges and alternate initiation times between trials. To control for timing strategies, participants were explicitly asked to avoid counting seconds or estimating time based on rhythmic activity (i.e., subvocalization, tapping and breathing).

Sequencing (Se): Participants performed two different complex sequences, alternating between trials. Both sequences involved non-adjacent finger movements: 2–4–3–5–3–4 and 2–5–3–4–3–5. However, the timing of initiation followed the simple condition in which participants initiated movements within a relatively large time range, from 5 to 12 s following the end of the previous sequence. This required participants to attend closely to the sequencing of movements, to perform the correct complex sequences alternating between trials.

In this way, the demand on motor timing and motor timing precision were higher in Timing condition than in Simple and Sequencing conditions. Conversely, the complexity of movement sequences and demand on processing of sequencing order were higher in Sequencing than in Simple and Timing. Crucially, because both the Timing and the Sequencing conditions involve alternating between complex patterns trial-by-trial, both should involve similar working memory demands. Direct comparisons between Timing and Sequencing conditions should therefore not be confounded by differences in cognitive or working memory demands.

We conducted pilot testing in which we varied the width of the temporal ranges for movement initiation and the order of the movements within sequences so that subjective task difficulty, as evaluated by 12 young healthy volunteers, was approximately
matched between Timing and Sequencing conditions. Therefore the repetitiveness of the task and the general level of difficulty were balanced across the Timing and Sequencing conditions, as assessed in pilot testing.

**Procedure**

The experiment involved two sessions, one with EEG recording and one with fMRI recording. In both sessions, the right hand was positioned palm down resting on a keyboard and fingers were positioned over the respective target keys, so that each key could be pressed by a simple flexion of the finger without lateral finger and wrist movements. Participants watched a fixation cross on a screen directly in front of them (in the EEG session) or through a mirror (in the fMRI session) and were instructed to avoid any eye or body movement other than required finger movements.

In the EEG session, each condition was divided into six blocks of 24 correct trials each, separated by short breaks. The order of conditions was counterbalanced across subjects with the latin square (three possible orders: ABC, BCA, CAB). Error trials were indicated to participants by visual feedback and excluded from subsequent analyses. Before the recording, participants performed a practice session consisting of one block for each condition.

In the fMRI session, the task conditions were performed in 1-min blocks (approximately eight trials) preceded by 15-s rest periods. The session involved three fMRI runs, with each run consisting of two blocks of each condition. The condition order within runs was counterbalanced across subjects with the latin square (three possible orders: ABC, BCA, CAB). To avoid extraneural activity related to processing error feedback, no feedback was presented in the scanner. To ensure that participants could adequately perform the alternating timing and sequencing patterns without feedback, the fMRI session was preceded by an extensive practice involving the EEG session, always recorded less than 2 weeks before, and a practice session of eight correct sequences for each condition performed immediately before the fMRI session.

**fMRI recording and analyses**

Data were acquired on a whole-body 3 Tesla Siemens Trio magnetic resonance imaging (MRI) scanner (Siemens Medical System, Germany) equipped with a standard quadrature head coil. Head motion was minimized by placing tight but comfortable foam padding around the subject’s head. For fMRI, Gradient echo-EPI images were acquired (TE = 40 ms, 64×64 matrix at 2.97×2.97 mm resolution, 26 axial slices with slice thickness = 3 mm and TR = 2010 ms). Two hundred twenty volumes were acquired per scanning run. The first four volumes of each scan were discarded to obtain stable magnetization. Structural MRI images were also acquired for each participant.

Image processing and statistical analyses were performed using Statistical Parametric Mapping (SPM2; Wellcome Department of Imaging Neuroscience, London, UK). Functional images were realigned with the first image of each sequence used as reference, spatially normalized to the MNI-152 reference image of SPM2, and spatially smoothed with a 6-mm full width half-maximum isotropic Gaussian kernel. Although experimental conditions in this study were blocked, the fMRI data was analyzed using an event-related model,
specifying the onsets of each finger movement sequence in each condition, and including temporal derivatives. This was done to account for any possible differences in the overlap of BOLD responses depending on the timing between successive movements, which might otherwise confound differences between conditions.

For group analysis, random effects analyses were performed using single-sample t-tests within SPM2. Firstly, group activation maps for each task condition compared with the resting baseline were calculated (Simple-Rest, Timing-Rest, and Sequencing-Rest) using a strict voxel-level statistical threshold of P<0.05 FWE corrected for multiple comparisons. Next, contrasts between task conditions were calculated (Timing-Simple, Sequencing-Simple, Timing-Sequencing, Simple-Timing, Simple-Sequencing) using a cluster-level threshold P<0.05 corrected for multiple comparisons (with clusters defined by voxel-level threshold P<0.01). Brodmann areas corresponding to the cortical activations were derived using the Brodmann Areas template image within MRICro (Tzourio-Mazoyer et al., 2002).

EEG recording and analyses

Electroencephalogram (EEG), electrooculogram (EOG) and electromyogram (EMG) were recorded using Synamps2 amplifiers (NeuroScan, El Paso, Texas, USA) and analyzed off-line with Scan 4.3 software. A Quick-cap electrode helmet was used to record EEG from 64 Ag/AgCl electrodes (Fp1, Fpz, Fp2, AF3, AF4, F7, F5, F3, F1, Fz, F2, F4, F6, F8, FT7, FC5, FC1, FC2, FC6, FC4, FT8, T7, T5, C3, T3, Cz, C2, C6, C4, T8, M1, TP7, CP5, CP3, CP1, CPz, CP2, CP6, CP4, CP6, TP8, M2, P7, P5, P3, P1, Pz, P2, P4, P6, P8, PO7, PO5, P03, PO2, P04, PO6, PO8, CB1, O1, O2, O2, CB2), according to the widening International 10/20 System with Cz’ (rostral to Cz) as reference. EEG was re-referenced offline to linked mastoids. Ag/AgCl electrodes were placed supra- and sub-orbitally to the right eye and 1 cm external to the outer canthus of each eye to record the vertical and horizontal movements in the EOG. The EMG activity was recorded using a pair of Ag/AgCl bipolar surface electrodes positioned over the right extensor digitorum muscle. The data were digitized at 500 Hz using 24-bit A/D converters. Lowpass filter was applied at 50 Hz for EEG and EOG recording and at 100 Hz for the EMG. The EMG signal was digitally re-filtered offline to 1–100 Hz and rectified. The analysis time window covered 2500 ms before and 500 ms after the first key press, using the first 500 ms as baseline. After blink artifact correction (Semlitsch et al., 1986) an artefact rejection threshold at ±60 μV was applied.

After the average epoch was obtained for each condition and each electrode, the recording sites were grouped into eight regions: left frontolateral (F7, F5, FT7, FC5), frontomedial (F1, Fz, F2, FC1, FC2, F2), right frontolateral (F8, F6, FT8, FC6), left centrolateral (C5, C3, CP5, CP3), centromedial (C1, Cz, C2, CP1, CPz, CP2), right centrolateral (C6, C4, CP6, CP4), left parietal (P5, P3, P1, PO7, PO5, PO3) right parietal (P6, P4, P2, PO8, PO6, PO4).

The epoch length before the movement not including the baseline interval (from -2000 to 0 ms) was divided into 10 non-overlapping 200 ms segments, from segment 1 (from -2.0 to -1.8 s) to segment 10 (from -0.2 to 0 s). The average across all time points in each segment was used as measure of readiness potential in the statistical analysis.

Three-way repeated-measures ANOVA was performed using a 3×10×8 experimental design: Condition (Simple, Timing, Sequencing) Time (10 segments) Region (IFL: frontal lateral, FM: frontomedial, rFL: right frontolateral, ICL: left centrolateral, CM: centromedial, rCL: right centrolateral, IPA: left parietal, rPA: right parietal). When appropriate, the Greenhouse–Geisser correction was used. Post-hoc comparisons were made using the Newman–Keuls test.

Behavioral analyses

For both the fMRI and the EEG sessions, three performance indices were computed in each task condition: The mean time interval between sequences, measured from the last movement of a sequence to the first of the next sequence; the mean inter-movement interval, measured as the time between button presses within sequences; and the mean percentage errors. A trial was considered correct if it met the requirements for both the timing of initiation and sequence order. Separate repeated-measures ANOVAs were performed including three levels of Condition (Simple, Timing, Sequencing), for data recorded during both fMRI and EEG sessions. When appropriate, the Greenhouse–Geisser correction was used. Post-hoc comparisons were made using the Newman–Keuls test.

Results

Behavioral results

In the EEG session, participants performed more errors in Timing (26±9.3%) and Sequencing (16.2±6.9%) conditions than in the Simple condition (8.2±4.8%) (main effect Condition: F(2, 32) = 71.54, P<0.05). Moreover, the mean error percentage was higher in the Timing condition than in the Sequencing condition.

The means and standard deviations of time intervals between sequences are shown in Fig. 2. As can be seen, the difference between intervals for odd and even sequences in the timing condition reflects the trial-by-trial alternation between short and long intervals. In the fMRI session, in the absence of error feedback to participants, the intervals between sequences tended to be longer than that required by instructions. However, as can be seen in Fig. 2B, the mean intervals for odd and even sequences for the timing condition clearly showed that participants still alternated between short and long intervals. Therefore, we reclassified timing errors in the fMRI session. Trials were counted as errors if, considering three consecutive sequences, there was no alternating pattern in the timing of sequence initiation. Error trials for all conditions were lower than 10% (Simple: 2.3±3.8%; Timing: 6.7±7.5%; Sequencing: 6.7±6.0%).

In the fMRI session, the overall mean interval between sequences was shorter for Timing (mean: 7466±1135 ms) than Simple (mean: 9117±1728 ms) and Sequencing conditions (mean: 9067±1591 ms; main effect Condition: F(2, 34) = 29.1, P<0.05). The same effect was found for the EEG session (Simple: 7325±658 ms; Timing: 6384±205 ms; Sequencing: 6788±673 ms; Main effect Condition: F(2, 32) =
Moreover, in the EEG session, intervals in the Simple condition were longer than in the Sequencing condition.

In both sessions, the mean inter-movement interval between button presses was longer in the Sequencing condition than in the other two conditions (fMRI: Simple 291±122 ms; Timing 284±118 ms; Sequencing: 330±117 ms; Main effect Condition: $F(2, 34) = 14.61, \varepsilon = 0.13, P < 0.01$. EEG: Simple 267±74 ms; Timing 266±68 ms; Sequencing 291±75 ms; Main effect Condition: $F(2, 32) = 8.99, \varepsilon = 0.69, P < 0.01$).

It is possible that cognitive demand in the different tasks may have changed with practice, which may have given rise to differences in performance depending on the order in which participants completed each of the three conditions. To check for such order effects, we conducted further analyses on performance accuracy including condition order (the three counter-balanced order groups) as a factor. For both EEG and fMRI sessions, there were no significant main effects or interactions involving the order of conditions ($P > 0.05$), indicating that performance accuracy did not vary depending on the order in which conditions were conducted Fig. 3.

**fMRI results**

Activations were first tested for each condition relative to baseline. Several brain regions revealed significant activation common to all conditions of movement. Bilaterally activation was found in the medial premotor cortex (PMC) including the SMA and the cingulate motor area, the lateral premotor area, inferior parietal gyrus, supramarginal gyrus (SMG), insula and putamen. In the left hemisphere activity was found in the precentral and postcentral sulcus, including sensorimotor cortex (SMC), and in the superior parietal gyrus and in thalamus. Moreover right superior parietal gyrus (SPG) was activated specifically in the Sequencing condition Fig. 4.

We then directly compared activation between each of the three movement conditions. Areas showing significant differences between conditions are shown in Tables 1 and 2.

When the Sequencing condition was compared directly with both Simple and Timing conditions (Table 1), the Sequencing condition showed greater activation in the lateral premotor area, inferior and superior parietal lobe, all bilaterally; cingulate motor area and supplementary motor area. No other differences were found related to these comparisons.

When the Timing condition was compared directly with both Simple and Sequencing conditions (Table 2), the Timing condition showed greater activation in the right superior frontal and medial orbito-frontal cortex involving Brodmann areas 10–46 and 11–47. The effect was visible but sub-threshold also for the left superior frontal cortex areas 10–46 (Timing–Simple: $x = -30, y = 62, z = 10$, Z score = 2.41; Timing–Sequencing: $x = -22, y = 64, z = 16$, Z score = 2.70). Additionally, in the
contrasts of Simple compared with Sequence condition (Table 2 B). Together, the source of the somewhat unexpected difference in the second condition was clearly present for all conditions of the task.

In agreement with the Readiness Potential (RP) topography, ANOVA showed main effects of Region \( F(7,112) = 13.28, \ e = 0.44, P < 0.01 \) and Time \( F(9,144) = 19.72, \ e = 0.13, P < 0.01 \), indicating that the readiness potential amplitude was larger over frontomedial and centromedial regions than over frontotemporal, centrolateral, and parietal regions, and that it increased from 2 s before the movement up to the time of movement initiation (from segment 1 to segment 10). Moreover, the significant interaction Time Region \( F(63,1008) = 7.53, \ e = 0.05, P < 0.01 \) showed that the increase of readiness potential amplitude over time was greater in the frontomedial and centromedial regions than the other regions.

Crucially, the amplitude of the readiness potential was modulated by the different task conditions. Increasing the movement timing demand and movement sequencing demand affected cortical premovement activity during different time intervals prior to movement and over different scalp regions. This effect was shown by the significant three-way interaction Time Region Condition \( F(126,2016) = 2.47, \ e = 0.07, P < 0.05 \).

The effect of motor timing was mainly present in the left and right frontotemporal regions for a period that included the early and late components of the readiness potential. Indeed, the RP recorded over the left and right frontotemporal regions showed significantly larger amplitude in the Timing condition than in the Simple and Sequencing conditions over time intervals from 1200 ms before movement to the time of

### Table 1

Activation differences for Sequence compared with Simple and Timing conditions (cluster-level threshold \( P_{\text{FWE}} < 0.05 \)).

<table>
<thead>
<tr>
<th>Region</th>
<th>( x )</th>
<th>( y )</th>
<th>( z )</th>
<th>( Z ) score</th>
<th>( x )</th>
<th>( y )</th>
<th>( z )</th>
<th>( Z ) score</th>
</tr>
</thead>
<tbody>
<tr>
<td>L SMA/CMA (BA 32)</td>
<td>-18, 44</td>
<td>4.03</td>
<td>-8, 54</td>
<td>4.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L precentral, premotor (BA 6)</td>
<td>-22, 0, 54</td>
<td>5.01</td>
<td>-28, -6, 52</td>
<td>5.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R precentral, premotor (BA 6)</td>
<td>-58, 8, 38</td>
<td>4.37</td>
<td>-60, 6, 34</td>
<td>4.77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L superior parietal (BA 7)</td>
<td>-28, -52, 52</td>
<td>3.94</td>
<td>24, -10, 52</td>
<td>4.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L inferior parietal (BA 39-40)</td>
<td>-18, -70, 50</td>
<td>4.75</td>
<td>-26, -56, 62</td>
<td>4.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R superior parietal (BA 7)</td>
<td>-40, -40, 50</td>
<td>4.54a</td>
<td>-40, -36, 46</td>
<td>4.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L inferior parietal (BA 39-40)</td>
<td>-30, -50, 56</td>
<td>4.12</td>
<td>32, -48, 52</td>
<td>4.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R inferior parietal (BA 39-40)</td>
<td>-20, 18, 28</td>
<td>4.00</td>
<td>-20, -52, 62</td>
<td>4.55</td>
<td></td>
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</tbody>
</table>

### Table 2

Left: Activation differences for Timing compared with Simple and Sequence conditions; Right: Activation differences for Simple compared with Sequence conditions (all cluster-level threshold \( P_{\text{FWE}} < 0.05 \)).

- R superior frontal (BA 10–46): \( x = 20, y = 54, z = 10 \)
- R medial orbito frontal (BA 11–47): \( x = 32, y = 54, z = -6 \)
- R superior parietal (BA 7): \( x = -18, y = -70, z = 50 \)
- L inferior parietal (BA 39–40): \( x = -60, y = -52, z = 44 \)
- L superior parietal (BA 7): \( x = -50, y = -52, z = 56 \)
- R angular gyrus (BA 39): \( x = -64, y = -52, z = 40 \)
- L superior frontal (BA 9): \( x = 12, y = -96, z = 28 \)
- L anterior cingulum (BA 25): \( x = -22, y = 56, z = 38 \)
- L posterior cingulum (BA 23): \( x = -2, y = -42, z = 32 \)

The contrast of Simple–Sequencing revealed greater activation in the same parietal network as the contrast of Timing–Sequence, involving the bilateral inferior parietal lobe and angular gyrus, right superior occipital lobe, left superior frontal area, left anterior and posterior cingulum.

There were no areas showing greater activation for Simple compared with Timing condition.

### EEG results

Movement-related potentials from 15 electrodes are shown in Fig. 5. A slowly increasing negative potential starting from 1.5 to 2 s prior to the movement and with maximum amplitude over the frontocentral sites was clearly present for all conditions of the task.

Fig. 4. Upper part: Contrasts for timing condition compared with simple and sequencing conditions show activation in the right dorsal- (rDLPFC) and ventral-prefrontal cortex (rVLPFC). Lower part: Contrasts for sequencing condition compared with simple and timing conditions show activation in the supplementary motor area/cingulate motor area (CMA), lateral premotor area (PMA), inferior (IPL) and superior parietal lobe (SPL) (cluster-level threshold \( P_{\text{FWE}} < 0.05 \)).
movement initiation. This effect was shown in comparisons between conditions in segments 3, 5, 6, 7, 8, 9 and 10. RP in the Timing condition was significantly higher than in the Simple condition over the left frontal lateral region at times 5, 6, 9, 10, and over the right frontal lateral region at times 5, 6, 7, 8, 9, 10. RP was also significantly higher for the Timing condition than for the Sequencing condition over the left frontolateral region at times 3, 5, 7, 8, 9, 10 (all comparisons significant for $P < 0.05$). In a separate analysis, we ensured that any differences seen in the Timing condition are not merely caused by differences in the intervals between movements, by comparing task performance and readiness potentials between long and short intervals in the Timing condition (results of this analysis can be found in the supplementary results).

The effect of motor sequencing was mainly present during the late component of the readiness potential and it was widely distributed over the scalp. Indeed, over both central and parietal regions RP amplitude was significantly greater in the Sequencing condition than in the Simple and Timing conditions during the last 600 ms prior to movement (segments 8, 9 and 10, all comparisons significant for $P < 0.05$). RP in the Sequencing condition was higher than in the Simple condition over left, medial and right central regions and over left and right parietal regions at times 8, 9, 10, and at time 7 in the right parietal region. Moreover, RP was higher in the Sequencing condition than in the Timing condition over the same areas at times 9 and 10 and on the centromedial area at time 8.

Last, the RP in the Simple condition was higher than in the Timing and Sequencing conditions on the frontomedial region at times 3, 4, 5, 7, 8 (Fig. 6).

**Discussion**

The aim of the study was to examine the processes of timing of movement initiation and sequencing of submovements that both contribute to preparing for voluntary action and to examine how their contribution is reflected in pre-movement brain activity. Our data suggest that preparing an action involves multiple systems that independently process specific characteristics of movements and that rely on independent brain circuits. Indeed brain activity was differently modulated when processing demand was increased on motor timing and when it was increased on motor sequencing.

When complexity of the timing for movement initiation was greater, the earliest component of premovement activity was increased, with the readiness potential showing a greater amplitude more than 1 s prior to movement onset. This effect occurred only over the fronto-lateral electrode sites in the MRPs, a result that matches well with the fMRI data in which the motor timing condition was associated with greater activation in the lateral prefrontal cortex.

In contrast, the complexity of movement sequences affected brain activity only immediately prior to movement execution, as indicated by an increase in the late part of the readiness potential but not the early component. In fMRI data, this effect of motor sequencing complexity was associated with increased activity in premotor, precentral and parietal areas bilaterally.

**Differential processing in action preparation**

It is well accepted that readiness for action is not a single holistic process. Previous studies generally consider two major components: higher cognitive processes relating generally to movement planning and lower-level processes relating to movement execution (for a review, Shibasaki and Hallett, 2006). Our data further distinguishes between sub-processes during movement planning that relate to different aspects of the movement to be performed. Elaborating the timing for movement initiation and the sequencing of submovement order are crucial parts of the planning for action and appear as two processes that rely on different brain regions and occur at different times prior to movement onset. Similarly, in other studies, differences in brain activation have been reported when sequences are created based on movement order and when created based on a rhythmic structure (Bengtsson et al., 2004; Schubotz and von Cramon, 2001; Ullen, 2007). Basic movement parameters, sequence complexity, and action selection have been shown to rely on segregated cortico-basal ganglia circuits (Lehericy et al., 2006). These previous studies, together with our present results, clearly show that multiple processes contribute to action preparation, particularly related to the timing and sequencing of the movement to be performed.
Increasing the complexity and task demands on the timing for movement initiation involved a prolonged activation of the lateral prefrontal cortex, from the early period of premovement activity up to the time of movement initiation. The readiness potential amplitude increased in the timing condition over the left and right frontal regions compared with the sequencing and simple conditions. In the fMRI, the activity in the prefrontal cortex was significantly higher in the timing condition compared with the other two conditions in the right hemisphere. Also, the same timing effect was apparent in the left prefrontal cortex, although it did not reach statistical significance.

The fact that changes in prefrontal cortex activity were related specifically to the Timing condition and that the associated changes in
fronto-lateral activity in the readiness potential occurred over a specific time period relative to movement and were not simply sustained across the whole task suggest that the prefrontal cortex is specifically involved in the processing of time related to movement initiation. This interpretation is supported by two lines of evidence. First, studies of self-initiated movement preparation show that the right DLPFC is particularly involved in the free selection of the timing of movement initiation, a process that requires a decision on when to initiate the movement (Jahanshahi et al., 1995; Jenkins et al., 2000). Second, the right DLPFC is a key structure for cognitively controlled time estimation (Lewis and Miall, 2002, 2003). According to Lewis and Miall's model, DLPFC would be involved as an internal clock for time estimation when processing of time requires overt attention to the task, as occurs for discontinuous, unpredictable, supra-second time management (Lewis and Miall, 2003). Data on reflexive saccade inhibition suggests that prolonged DLPFC activity before movement is inhibitory (Gaymard et al., 1998). Therefore, the DLPFC may be important for both elaborating time under cognitive control and making the decision on when to move, withholding movement until the right moment. It should be noted that, although we instructed participants explicitly not to use counting or subvocalisation strategies for timing, as is standard in this field, we cannot be sure that no such strategies were ever used by participants, nor is it known how the role of the DLPFC in elaborating timing may vary under these different conditions.

The DLPFC is also known to be important for working memory and monitoring processes for supervising performance, as modeled within the Supervisory System framework (Shalllice, 2002; Shalllice and Burgess, 1998). DLPFC activation in the Timing condition, however, is unlikely to be due simply to greater complexity of the task or higher cognitive demands. Both complex tasks in our study (timing and sequencing) involve significant working memory load and increased attention to action compared with the simple condition, as participants were required to maintain an alternating pattern between two sequences/timings. However, activation of the right DLPFC, and increased amplitudes of early premovement activity over fronto-lateral sites, was specific to the timing condition and not observed for complex sequencing of movement. Furthermore, behavioural data showed no differences in performance accuracy depending on the order in which conditions were performed. Results therefore cannot be explained by any possible differences in cognitive demand that may have changed with practice across the different conditions.

Wiese and co-workers have suggested a working memory or attentional role of prefrontal cortex in self-initiated movement paradigms as they found that prefrontal activity related to their motor task was sustained throughout the whole task (Wiese et al., 2005). While there may have been similar underlying sustained activity in our task, it is also clear from the readiness potential data that activity over lateral prefrontal areas increased in the Timing condition specifically over the time interval from 1 s prior to movement, relative to the earlier baseline interval. Changes in lateral prefrontal activity associated with movement timing were therefore not only due to stable sustained activity across the whole task but reflected specific processes occurring regularly from 1 s prior to the initiation of the voluntary actions. Therefore, while the DLPFC is clearly important for working memory and cognitive control of attention, our results suggest that the DLPFC also plays an important role in determining the timing for movement initiation prior to voluntary action.

It is noteworthy that the effect of time processing on brain activity starts from the earliest stage of action preparation and lasts until the movement is performed. This result suggests that processing the time for movement initiation is an early process preceding the movement and a major contributor to the preparation for action and the readiness potential in self-initiated movement paradigms.

Lastly, we cannot exclude that the early readiness potential and DLPFC activity could represent a role in the intentional decision on when to initiate movement. Previous studies have shown that both left and right DLPFC are involved when actions are freely selected (Cunnington et al., 2006; Frith et al., 1991; Lau et al., 2004). Free selection of action has also been reported to have an effect on the early component (Dirnberger et al., 1998) and the late component of the readiness potential (Praamstra et al., 1995). Decisions on when to move and other intentional decision processes have never been directly compared and therefore whether they share common neural mechanisms is still an open question.

Motor sequencing

Complexity and higher task demands on ordering movements within sequences increased activity of medial premotor area, including cingulate motor area and supplementary motor area, lateral premotor area, inferior and superior parietal lobe, all bilaterally. The activation of this fronto-parietal circuit occurred only immediately prior to movement execution, in the period corresponding to the late component of the Readiness Potential.

The motor sequencing effect on the fronto-parietal circuit in our results is in line with other studies that have investigated this process (Bengtsson et al., 2004; Boecker et al., 1998; Catalan et al., 1998; Sadato et al., 1996a) and with the suggestion that frontal and parietal areas have an important role in movement selection and in planning the ordinal representation of actions (Bengtsson et al., 2004; Rushworth et al., 2001b). Rushworth and coworkers have suggested that premotor and parietal areas are crucial for shifting from the preparation of one movement to another (Rushworth et al., 2001b). This process could serve as the basis for creating abstract representations of the ordinal structure of sequences when fast sequences of movements are performed and the complete planning of the sequence is required before the initiation of the first movement (Kenneley et al., 2004).

We also found a separate parietal network involving inferior parietal lobe around the angular gyrus and superior occipital cortex that was more active for the simple repetitive sequence (in both Simple and Timing conditions) compared with the alternating complex sequences. Dirnberger and colleagues have suggested that, for repetitive movements, a motor trace of the previous movement may remain intact and may contribute to the preparation of the same movement when repeated, whereas alternating different movements requires a reset of the previous motor program and the full preparation of the new movement (Dirnberger et al., 2000). We suggest that the occipito-parietal activation found during simple repetitive sequences compared with the alternating complex sequences may relate to the maintenance of a trace of the previous motor program for the repetitive movements, and may therefore represent a type of motoric working memory process that can facilitate movement programming.

Planning and programming a series of movements is a crucial part of the higher cognitive functions that occur before processing for movement execution (Shibasaki and Hallett, 2006). Our results support the suggestion that complex processes of movement preparation or the programming of specific movement parameters, such as planning the ordinal structure of a movement sequence, occur only in a short period immediately preceding movement execution. Indeed, we have shown that the modulation of sequence complexity affects the late but not the early component of premovement activity, only within 600 ms of movement onset. Other studies have similarly shown that movement-specific factors, such as the degree of fine motor control required (Kitamura et al., 1993a), whether movements are sequential or simultaneous (Kitamura et al., 1993b), and the level of force production (Masaki et al., 1998), are all represented only in the late component of the readiness potential. These results provide strong evidence that complex planning of movement-specific parameters is a process occurring during the late stage of the preparation for voluntary action.
The readiness for action

While prefrontal and premotor parietal regions were differentially influenced by motor timing and motor sequencing, we must emphasize that these regions were still active in all movement conditions. As seen in Fig. 3, the network of brain regions involved in the voluntary actions appears very similar for all conditions, whether simple and repetitive or with complex sequencing or timing. All three conditions involved significant and strong activation in supplementary and cingulate motor areas, lateral premotor and inferior parietal regions, and in the putamen and thalamus. Premovement activity represented in the readiness potential was also highly similar for all movement conditions, with the typical maximum found in central midline sites that lie close to the SMA. This suggests that common processes of movement preparation are involved in all movement conditions and differences related to timing and sequencing conditions are only subtle.

We suggest that the preparation/readiness for action is, of course, not a simple process. Here we show that multiple brain regions contribute to the readiness for action, each contributing to different aspects of the movement to be performed. While the predominant neural activity appears to be similar across simple and complex actions, suggesting a common network involved in movement preparation, subtle differences in the activity of these regions can be seen over different time periods depending on the requirements of the intended action. As the complexity of movement timing increases, and attention is focused on the timing of movement initiation, the lateral prefrontal regions play a greater role and contribute to differences in the earliest component of premovement activity, up to 1 s prior to movement initiation. As the complexity of movement sequencing increases, and attention is focused on the ordering of submovements within the sequence, premotor and parietal regions play a greater role in the later period of movement preparation, immediately prior to movement initiation. It is the coordinated activity of these multiple regions, contributing to different aspects of the intended action over different time periods that are represented in premovement activity during the readiness for voluntary action.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2009.11.048.

References


