Motor skill learning requires the involvement and integration of several cortical and subcortical regions. In this study, we focus on how the functional connectivity of cortical networks changes with the acquisition of a novel motor skill. Using functional magnetic resonance imaging, we measured the localized blood oxygenation level-dependent (BOLD) signal in cortical regions while subjects performed a bimanual serial reaction time task under 2 conditions: 1) explicitly learning a novel sequence (NOVEL) and 2) playing a previously learned sequence (LEARNED). To investigate stages of learning, each condition was further divided into nonoverlapping early and late conditions. Functional connectivity was measured using a task-specific low-frequency coherence analysis of the data. We show that within the cortical motor network, the sensorimotor cortex, premotor cortex, and supplementary motor area have significantly greater inter- and intrahemispheric coupling during the early NOVEL condition compared with the late NOVEL condition. Additionally, we observed greater connectivity between frontal regions and cortical motor regions in the early versus late NOVEL contrast. No changes in functional connectivity were observed in the LEARNED condition. These results demonstrate that the functional connectivity of the cortical motor network is modulated with practice and suggest that early skill learning is mediated by enhanced interregional coupling.

Keywords: bimanual, coherence, dorsolateral prefrontal cortex, fMRI, supplementary motor area

Introduction

Complex motor sequence skills, such as typing or playing the piano, may be acquired with practice over a period of time, from minutes to days, weeks, or months, depending on the difficulty of the task. Such skill learning involves multiple cognitive processes and requires the integration of a broadly distributed network of cortical and subcortical regions (for review, see (Willingham 1998)). Whereas a number of imaging studies have examined the function of individual regions within the network during the process of motor learning, little is known about the interactions of the network as a whole. In this paper, we investigate learning-related changes in the interregional functional connectivity of the cortical motor network, using coherence analysis of functional magnetic resonance imaging (fMRI) data.

Studies of patients who have undergone callosotomies provide the most salient examples of the necessity of interregional connections for motor skill learning. Sperry (1968) reported that such patients exhibited deficits in learning novel bimanual tasks but were able to perform bimanual tasks learned prior to the surgery. Even after extensive practice, subjects whose anterior commissures were sectioned show less improvement on bimanual tasks compared with normal subjects (Preilowski 1972; Jeeves and others 1988). Thut, Halsband, Regard, and others (1997) examined intermanual skill transfer in patients with callosal agenesis; their improved nondominant to dominant transfer compared with normal subjects suggests a potential inhibitory influence of the corpus callosum in intermanual skill transfer. The idea that interhemispheric connectivity is a substrate of bimanual skill learning is further supported by neuroimaging studies. Recent electroencephalography (EEG) studies have investigated such interregional interactions during complex bimanual skill learning using task-related coherence. Andres and others (1999) and Serrien and Brown (2003) found increases in interhemispheric coupling within the cortical motor network during early learning stages, which was significantly reduced during later learning stages. Importantly, Andres reported that whereas the early interhemispheric coupling was increased for the bimanual task, no significant increase was observed for the unimanual task. The poor spatial specificity of EEG, however, does not allow one to assess the activity or connectivity of precise regions. Therefore, we adapted the bimanual sequence-learning task in the Andres'/Stark's study to a serial reaction time (SRT) task for an fMRI study.

The SRT task designed by Nissen and Bullemer (1987) is a behavioral task widely used to measure motor sequence learning. In the standard form of this task, subjects are presented with sequential visual cues, each corresponding to 1 of 4 key responses. Subjects are asked to respond to each cue as quickly and accurately as possible. Sequence learning is measured by a reduction in reaction time (RT) over the period of trials. Modifications to this task have been used to explore different processes of motor learning such as explicit versus implicit learning (Jenkins and others 1994; Grafton and others 1995; Hazeltine and others 1997) or response-based versus perceptual-based learning (Cohen and others 1990; Keele and others 1995; Koch and Hoffmann 2000). These studies have demonstrated that the pattern of learning-related activity within the motor network is influenced by a number of different properties of the task paradigm, such as the level of sequence awareness, the amount of stimulus–response compatibility, and the number of distracting tasks.

In this study, our aim was not to distinguish between networks associated with different learning processes under various conditions but rather to identify learning-related changes in the motor network that correspond to a simple motor learning paradigm, namely, a bimanual, explicit, response-based sequence-learning task. Therefore, our experimental design employs a variation of the SRT task intended to isolate such changes. First, subjects were presented with compatible, spatially relevant cues to encourage response-based learning (Koch and Hoffmann...
Second, to minimize complex interactions and heterogeneous networks associated with multiple tasks, our experimental design employed a single task paradigm rather than a dual-task paradigm as used by Grafton and others (1995). Third, to reduce the variability in sequence awareness and strategy use across subjects, all subjects were told that there was a sequence present and were asked to learn the sequence explicitly. Lastly, the bimanual sequence alternates between the 2 hands, modeled after the bimanual sequences used in the Andres’ study.

Control-based learning theory predicts that explicit sequence learning engages 2 processes, strategic learning and sequencing, and furthermore suggests that these 2 processes are supported by distinct brain regions, namely, the dorsolateral prefrontal cortex (DLPFC) and the supplementary motor area (SMA), respectively (Willingham 1998). Evidence from lesion and imaging studies strengthen these arguments. Patients with damage to the prefrontal cortex have difficulty performing goal-oriented or planned movements, whereas reflexive movements remain unimpaired (Pierrot-Deseilligny and others 2003). In contrast, patients with lesions of the SMA exhibit difficulty in performing multiple sequential motor tasks, whereas they were able to perform each individual task well. Additionally, patients with lesions in the SMA and/or premotor cortex (PM) have major difficulties reproducing temporal sequences; the most severe deficits were in reproducing rhythms from memory; however, patients were able to reproduce rhythms under auditory pacing (Laplane and others 1977; Halsband and others 1993). Neuroimaging studies have also demonstrated the relevance of these regions during explicit sequence-learning tasks. Tasks requiring explicit sequence learning have been shown to activate both the DLPFC (Grafton and others 1995; Honda and others 1998; Eliassen and others 2001) and the SMA (Honda and others 1998; Muller and others 2002; De Weerd and others 2003) in addition to a number of other cortical regions, including the PM and posterior parietal cortex (PPC). These results suggest that although the DLPFC and SMA are necessary for explicit sequence learning, they do not operate in an isolated fashion. Instead, they are integrated with an extended network of motor regions engaged by the learning process.

This study focuses on the interregional interactions during the explicit motor sequence-learning process. Recent advances in neuroimaging and multivariate analysis techniques have demonstrated the importance of examining the interactions between regions to understand how networks are engaged during cognitive processes (Friston and others 1993; McIntosh and Gonzalez-Lima 1994). Using a coherence analysis method recently developed for fMRI data (Sun and others 2004), we examine the functional connectivity of the motor network during bimanual motor sequence learning. There are 2 specific goals of the study. First, we aim to replicate the EEG results from the Andres’ study for this task paradigm and to extend the results by identifying specific regions within the motor network that have increased interhemispheric connectivity during early learning. Second, we aim to examine the interactions between the DLPFC and SMA with other regions within the motor network during explicit sequence learning.

Methods

Subjects and Behavioral Task

Fourteen right-handed subjects (4 females, aged 19-29 years, mean age = 23.5 years) participated after giving informed consent according to procedures approved by the University of California. The subjects reported no history of neurological or psychiatric disorders and were taking no medications at the time of the study.

Training

Prior to scanning, subjects were trained explicitly on 1 of 2 bimanual sequences: sequence A, denoted by L4-R2-L3-R4-L5-R3-L2-R3, or sequence B, denoted by L5-R3-L2-R5-L4-R2-L3-R4. Here, the letter signifies the hand (R = right and L = left), and the number signifies the finger (2 = index finger, 3 = middle finger, 4 = ring finger, and 5 = fifth finger). Both sequences were 8 keystrokes long, alternating between the left and right hands, and engaging each of the 8 fingers exactly one time.

During training, the subject was seated comfortably in a dimly lit room. The subject’s hands were positioned on a 5-fingered response box such that each finger aligned with a single key. The training sequence was presented to the subject as a series of 8 consecutive visual cues indicating the key to press. During the presentation of the sequence, a cue was presented to the subject every 725 ms for a total sequence time of 5800 ms. The subject was instructed to respond as accurately as possible within the response window for each cue (725 ms). A sequence was considered correct only when all 8 key presses were performed correctly. The same sequence was repeated 20 times within a set, with a 2-s intersequence interval. Subjects were trained until they were able to complete a set of sequences with an accuracy of at least 85%. Training sessions consisted of an average of 4 sets (80 sequence trials).

Magnetic Resonance Imaging Scanning

In the magnetic resonance imaging (fMRI) scanner, five 8-min functional runs were acquired for each subject using a mixed block/event-related paradigm (Visscher and others 2003). The runs were composed of 4 condition blocks (LEARNED, NOVEL, RANDOM, and REST), each presented twice in a pseudorandom order, counterbalanced across subjects. Each block began with an instructional cue indicating the type of block. Within each of the LEARNED, NOVEL, and RANDOM condition blocks, sequences were presented with visual cues as in training (725 ms per key press, 5800 ms per sequence), but with a pseudorandomized intertrial interval of 2.2, 4.4, or 6.6 s (Fig. 1). During the LEARNED condition, subjects were presented with the same sequence that they learned during the training session (e.g., sequence A). During the NOVEL condition, subjects were presented with the alternate sequence (e.g., sequence B), which was novel to them at the beginning of the scan session. Subjects were instructed to learn the repeating sequence across the blocks. During the RANDOM condition, subjects were presented with a new sequence for each trial. Subjects were instructed to play all sequences as accurately as possible. Five sequences were presented in each block for a total block length of 58 s. During the REST block, also of length 58 s, subjects were presented with a centered fixation cross. For this condition, subjects were instructed not to perform or rehearse any of the sequences. Subjects received accuracy and timing information at the conclusion of each condition block. The results from the LEARNED, NOVEL, and REST conditions are presented in this paper.

The stimuli were designed and presented using Eprime presentation software (www.pstnet.com). They were then back projected onto a custom-designed, nonmagnetic projection screen that the subject viewed via a mirror. Responses were collected using a pair of 5-fingered magnetic resonance (MR)-compatible keyboards.

MRT Data Acquisition

All images were acquired with a 4-T Varian INOVA MR scanner (www.varianinc.com) and a TEM-send and receive RF head coil (www.mr-instruments.com). Functional images were acquired using a 2-shot gradient-echo planar image sequence with a repetition time of 543 ms per half k-space, an echo time of 28 ms, and flip angle of 20°, resulting in 432 total volumes acquired per run (864 after time interpolation). Each volume, covering the top of the brain, consisted of ten 5-mm-thick axial slices with a 0.5-mm interslice gap. Each slice was acquired with a 22.4 cm2 field of view with a 64 × 64 matrix size, resulting in an in-plane resolution of 3.5 × 3.5 mm. High resolution (0.875 × 0.875 mm) in-plane T1-weighted anatomical images were also acquired using a gradient-echo multislice sequence for anatomical localization. Finally, MPFlash 3-dimensional T1-weighted scans were
acquired so that functional data could be normalized to the Montreal Neurological Institute (MNI) atlas space.

**MRI Data Analyses**

**Preprocessing**

Functional images acquired from the scanner were reconstructed from k-space using a linear time-interpolation algorithm to double the effective sampling rate and corrected for slice-timing skew using temporal sinc interpolation. Images were then corrected for movement using rigid body transformation parameters and smoothed with an 8-mm full width at half maximum (FWHM) Gaussian kernel using SPM2 (www.fil.ion.ucl.ac.uk/spm/).

**Univariate Analyses**

To model task-related activity, we used the canonical hemodynamic response function (HRF) (Josephs and others 1997) convolved with independent variables for the onset and duration of each sequence. Here, the duration of each sequence was modeled as an epoch of 5800 ms. These covariates were entered into the modified general linear model for analysis. Parameter estimates, reflecting the percent signal change relative to baseline, were estimated for each covariate. Statistical parametric maps (t-statistics) of contrasts were generated for the group after individual subject data were resampled to 2-mm isotropic voxels, smoothed by a 4-mm FWHM Gaussian kernel, and spatially normalized into the standard MNI atlas space using SPM2. Group-level random-effects analyses were performed using a nonparametric approach, statistical nonparametric mapping (SnPM), applying permutation analyses on the entire volume, using a threshold of \( P < 0.05 \), controlling for familywise error (FWE).

**Coherence Analysis**

To identify networks of functional connectivity for each region of interest (ROI), we generated coherence maps using the task-specific coherence between reference voxels, or seeds, and all other regions in the brain. To identify changes in functional interactions with task, we contrasted the task-specific coherence maps. This method is discussed in detail in Sun and others (2004) and is outlined in 4 steps below.

**Selection of reference voxels** For each subject, we selected reference or seed voxels within our ROIs, the right DLPFC (rDLPFC) and the SMA. Additionally, reference voxels from other ROIs in the motor network were selected based on evidence in the literature and results from the univariate analysis. These were located in the primary sensorimotor cortex (S1/M1), dorsal premotor cortex (dPM), and PPC. For each of the ROIs, anatomical masks were defined in the subjects’ native space. The DLPFC mask was located in the middle frontal gyrus, anterior to the precentral sulcus. The SMA mask was located on the medial wall, dorsal to the cingulate gyrus, and between the central sulcus and the vertical plane through the anterior commissure (Picard and Strick 2001). The S1/M1 mask included cortex adjacent to the central sulcus, extending anteriorly to the midline between the central and precentral sulci, posteriorly to the midline between the central and postcentral sulci at the “knob,” but also extending dorsally and ventrally several millimeters to include the entire motor hand area (Yousry and others 1997). The dPM mask extended from the midline between the central and precentral sulci, anteriorly to the junction of the superior frontal sulcus and the precentral sulcus. To exclude ventral premotor regions, the dPM mask included only cortex dorsal to the inferior frontal sulcus (Picard and Strick 2001). The PPC mask included cortex in the superior parietal lobe posterior to and inclusive of the postcentral sulcus and inclusive of the intraparietal sulcus (IPS) at the junction of the 2 sulci (Simon and others 2002). All masks were nonoverlapping. Within each masked region, we identified a seed ROI as the voxel with the most task-related activity, based on univariate statistics, as well as all the significant voxels in the 6-mm radius sphere surrounding the voxel. Motor seeds were selected within the left (dominant) hemisphere only because not all subjects had significant task-related activity for all seed regions in the right hemisphere.
Generation of condition-specific time series. To generate condition-specific time series, the data from each voxel were segmented into condition blocks. Each segment was mean centered, windowed using a 4-point split-cosine bell (Bloomfield 1976), and concatenated with segments of the same condition. Windowing reduces spectral leakage from any discontinuities introduced by segmenting and concatenating the time series. The LEARNED, NOVEL, and REST condition-specific time series each had a total of 1040 data points. Additionally, the first 4 blocks (416 time points) of the NOVEL time series were extracted to create the time series for the early NOVEL condition, and the last 4 blocks were extracted to create the time series for the late NOVEL condition. The early and late LEARNED time series were extracted in the same way.

Estimation of condition-specific coherence maps. Coherence estimates were computed using a fast Fourier transform algorithm implemented in Matlab 6.5 (http://www.mathworks.com). We used Welch’s periodogram averaging method to estimate the condition-specific coherence of each seed ROI with all other voxels in the brain (using a 64 point discrete Fourier transform, Hanning window, and overlap of 32 points). We then generated coherence maps for each seed ROI for each condition using the estimate of the band-averaged coherence within the bandwidth of the HRF (0-0.15 Hz band) (Sun and others 2004).

Contrasts of condition-specific coherence maps. To identify changes in functional connectivity across conditions, we contrasted the coherence maps. We applied an arc-hyperbolic tangent transform to the coherency, as described in Rosenberg and others (1989), so that the difference of the coherency magnitudes approaches a zero-centered normal distribution. This transformation allows us to apply a parametric random-effects group analysis (a 2-tailed, 1-sample t-test) on the difference maps to determine regions with significantly different connectivity for each seed across conditions. Group-level random effects were performed using a permutation method (SnPM), with a cluster threshold of k > 100, P < 0.05, corrected for multiple comparisons (FWE).

Results

Behavioral Results

The mean accuracies during the early LEARNED condition and late LEARNED condition were 92 ± 10% (standard deviation) and 90 ± 12%, respectively, with an overall average for the LEARNED condition of 91 ± 10%. In comparison, the accuracies during the early NOVEL condition and late NOVEL condition were 79 ± 13% and 86 ± 10%, respectively, with an overall average for the NOVEL condition of 82 ± 9%. The accuracy data were transformed using an arcsine-root transform and entered into a within-subjects analysis of variance (ANOVA) to examine the effects of pretraining (NOVEL vs. LEARNED) and practice (early vs. late) on mean accuracy. We found a significant main effect of pretraining (F = 12.042, P < 0.005) and a significant interaction between practice and pretraining (F = 6.520, P < 0.05). Post hoc analyses revealed that the mean accuracy was significantly greater for the late NOVEL condition compared with the early NOVEL condition (t = 2.702, P < 0.05), whereas there was no significant difference in accuracy between the early and late LEARNED conditions (t = 1.236). Additionally, the accuracy for the early NOVEL condition was significantly less than the accuracy for the early LEARNED condition (t = -4.638, P < 0.0005), whereas there was no significant difference between the late NOVEL and late LEARNED conditions (t = -1.176) (Fig. 2a).

The average RT for each key press during the LEARNED condition was 237 ± 49 ms, and the average RT for the NOVEL condition was 314 ± 27 ms. The RT for the early and late LEARNED conditions were 269 ± 40 ms and 205 ± 60 ms, respectively, whereas the RT for the early and late NOVEL conditions were 358 ± 28 ms and 272 ± 40 ms, respectively. A 2-way ANOVA revealed a significant main effect of pretraining (F = 48.156, P < 0.0005) and of practice (F = 60.698, P < 0.0005), with no significant interaction. Post hoc analyses revealed that there was a significant decrease in RT between the early and late NOVEL conditions (t = 6.788, P < 0.0005) and a significant decrease between the early and late LEARNED conditions (t = 5.808, P < 0.0005). Additionally, there was a significant difference between the early NOVEL versus LEARNED conditions (t = 7.390, P < 0.0005) as well as the late NOVEL versus LEARNED conditions (t = 4.710, P < 0.0005) (Fig. 2b).

Imaging Results

Our main interest in this study was to investigate changes in the motor network associated with motor sequence learning. Therefore, our results focus on the main effect of task during the NOVEL condition and differences between the early and late NOVEL condition. For completeness, we also examine
changes in the network during the LEARNED condition as well as the NOVEL versus LEARNED contrast.

Main Effect of Task: The NOVEL Condition
The univariate statistical parametric map of the NOVEL condition (compared with the baseline REST condition) revealed regions of activity typical for a bimanual visuomotor response task. During the NOVEL condition, S1/M1, dPM, and IPS were activated bilaterally, and SMA, cingulate motor area (CMA) (Picard and Strick 2001), and precuneus were activated medially. In addition, there were bilateral activations of the DLPFC (Brodmann area 46), which were stronger on the right side (Fig. 3A).

Coherence maps for the S1/M1, dPM, PPC, SMA, and DLPFC seeds revealed networks of regions that have increased functional connectivity with the seed during the NOVEL condition compared with the REST condition. All motor seeds (S1/M1, dPM, PPC, and SMA) revealed similar, but nonidentical, maps of functional connectivity during the NOVEL condition (Fig. 3B–E). The region with the greatest task-related changes in interregional connectivity was in the PM. The dPM seed showed increased coherence with the contralateral dPM, ipsilateral SMA, bilateral S1/M1, and PPC. Reference regions within S1/M1, SMA, and IPS also had greater inter-and intrahemispheric connectivity during the NOVEL condition compared with the REST condition. There were no regions with significantly greater connectivity with the rDLPFC seed during the NOVEL condition compared with the REST condition. Additionally, there were no regions with greater coupling during the REST condition compared with the NOVEL condition.

Learning-Related Changes in the NOVEL Condition: Contrast of Early and Late NOVEL
To investigate the NOVEL condition further, we compared the first 4 blocks with the last 4 blocks of the NOVEL condition. Whereas there were no significant differences in the mean level of activity between the early and late NOVEL conditions, the contrast of connectivity maps for the early versus late NOVEL conditions exposed a large network of regions that have greater interactivity during the early learning stage compared with the late learning stage ($k > 100$, $P < 0.05$, corrected [FWE]). The reference region in the primary motor cortex revealed the greatest network of interhemispheric connectivity, including bilateral S1/M1, dPM, and PPC. In addition, there was greater coupling between the S1/M1 seed and medial regions (SMA and pre-SMA) during early learning compared with later learning (Fig. 4A). The dPM coherence contrast map also revealed a network of interregional coupling enhanced during early learning; the most significant regions were located in S1/M1 and PM ipsilateral to the seed, but the network also included the contralateral S1/M1 and PM and the SMA (medial). The PPC and SMA seed regions also had increased coupling with the ipsilateral PM (PPC seed) and S1/M1 (dPM and SMA seeds) and the right superior parietal lobule. Lastly, the rDLPFC had greater coherence with the right PM during the early NOVEL condition compared with the late NOVEL condition. There were no regions that showed greater coupling during the late NOVEL condition compared with the early NOVEL condition.

As a control, we also compared the NOVEL and LEARNED conditions, and the early and late LEARNED conditions. No significant differences in activity or connectivity were found. Additional post hoc analyses contrasting the early LEARNED to the late NOVEL condition also revealed no significant differences in activity or connectivity.

Discussion
Our data show that intra- and interhemispheric connectivity are enhanced during the early phase of bimanual skill acquisition. Increased interhemispheric coupling during early learning was predicted by studies that documented bimanual learning deficits in patients with lesions of the corpus callosum (Sperry 1968; Preilowski 1972). Although such lesion studies provide information about the necessity of interhemispheric cortical connections, they do not provide temporal or spatial specificity of the connectivity. Using coherence analysis of fMRI data, we were able to investigate the dynamic nature of the connectivity associated with specific ROIs.

Our results show greater coupling between the primary sensorimotor cortex reference region and contralateral motor regions during the early NOVEL condition compared with the late NOVEL condition. These results are in agreement with EEG studies that report initial increases in the interhemispheric alpha-band coherence, which later decrease with practice (Andres and others 1999; Serrien and Brown 2003). Serrien

![Figure 3](image-url)
and Brown (2003) further report that this decrease in coherence with practice was specific to the electrodes overlying the primary sensorimotor cortex (C3–C4). Moreover, these practice-induced changes within the S1/M1 network are consistent with other studies that demonstrate capability of the human primary motor cortex to reorganize rapidly with practice (Pascual-Leone and others 1995; Classen and others 1998). Together, these results provide compelling evidence of the dynamic nature of the coupling between the primary cortex and other motor cortical regions, and support the hypothesis that S1/M1 is an integral component within a dynamic motor network, in the acquisition of novel bimanual skills.

Additionally, our results show that the connectivity between the motor network and higher order cognitive regions is enhanced during early learning. Specifically, we observed an increased interaction between the rDLPFC and the right PM. This increased interaction occurred only during the early NOVEL condition, when subjects continued to improve in accuracy, and was not present during the late NOVEL or LEARNED conditions, when subjects achieved maximal accuracy. These results corroborate a number of imaging studies that have associated activity in frontal areas with explicit motor learning (Grafton and others 1995; Thut, Halsband, Roelcke, and others 1997; Honda and others 1998; Eliassen and others 2001). In Eliassen and others (2001), activity in the prefrontal cortex was greatest during early learning phases and decreased as learning progressed. Such evidence is also consistent with observations that the prefrontal cortex is associated with monitoring, verification, and spatial response selection (Schumacher and D’Esposito 2002; Cabeza and others 2003; Schumacher and others 2003). In this context, our results suggest that such strategic learning mechanisms are important during explicit learning, and the coupling between the strategic network and the motor network provides a substrate for explicit motor learning.

In this study, motor seeds were selected within the left (dominant) hemisphere only because not all subjects had significant task-related activity for all seed regions in the right hemisphere. There is evidence that suggests similar results may be expected from the nondominant primary motor cortex. In an earlier study, we showed symmetric connectivity results associated with the dominant and nondominant primary motor cortices (Sun and others 2004). Additionally, Serrien and Brown (2003) identified a symmetric decrease in the EEG coherence in the electrodes overlying the primary sensorimotor cortex (C3–C4). To our knowledge, there have been no studies that specifically examine the motor learning-related differences in connectivity between dominant and nondominant regions; however, behavioral measures suggest that there may be a difference. Thut, Halsband, Regard, and others (1997) observed differences in the transfer of motor skills from the dominant to nondominant hand as compared with the transfer from the nondominant to the dominant hand, suggesting that the networks associated with motor learning may differ between the dominant and nondominant cortices.

After the initial learning phase, subjects continued to improve with practice, as demonstrated by a significant decrease in the RT between the early and late LEARNED conditions. During this later stage, which is described as postlearning, Honda and others (1998) reported that decreased RTs were correlated with increased activity in S1/M1 and SMA. The SMA and pre-SMA regions have also been identified as regions that are preferentially active when an overlearned motor sequence is generated from memory (Mushiake and others 1991; Halsband and others 1994). Additionally, the PM has been identified as a region associated with overlearning (Seitz and others 1994). In our study, we found no differences in the activity or connectivity of the SMA, M1, or PM during the late LEARNED task as compared with the early LEARNED task. This may be because the few trials within each condition did not provide enough power to measure a statistical difference between these 2 conditions. In addition, this task differs from other “overlearned” conditions in that the subjects are not required to retrieve the sequence from memory. Rather, it is presented to the subject in the same way as in the early LEARNED condition and the NOVEL conditions. Nevertheless, our results suggest that changes in the cortico-cortical coupling related to the postlearning process (during the LEARNED condition) may be less pronounced than the changes observed in the early learning process (during the NOVEL condition).

It is also a possibility that some postlearning processes may be mediated by subcortical structures such as the cerebellum and basal ganglia. Patients with damage to the cerebellum demonstrate little or no learning on SRT tasks as measured by RTs (Pascual-Leone and others 1993; Gomez-Beldarrain and others
1998). Similarly, patients with striatal damage are impaired on sequence-specific learning tasks, showing little to no improvement in response times for repetitive motor tasks (Harrington and others 1990; Knopman and Nissen 1991; Jackson and others 1995; Willingham and others 1996). Imaging studies are less conclusive, some studies have reported activity increases in the basal ganglia during late stages of sequential learning (Grafton and others 1992; Seitz and Roland 1992; Doyon and others 1996), whereas others have reported decreases (Grafton and others 1995; Jueptner and others 1997). In the cerebellum, late-learning-related decreases have been reported (Jenkins and others 1994; Jueptner and others 1997; Toni and others 1998). In this study, we focused on cortico-cortical interactions and did not collect data from subcortical regions; therefore, we were unable to directly measure corticossubcortical coupling.

Although we have highlighted several learning-related changes in intra- and interhemispheric interactivity, we observe few learning-related differences in activity. A possible explanation for the discrepancy between our results and those of other studies is that in this study the sequence was learned quickly (within 4 blocks) as measured by accuracy, thereby reducing the power of the NOVEL condition. Other studies that have found learning-related changes in activity have used tasks that extend the learning period, such as using trial-and-error learning or by providing a novel learning sequence for each block. Nevertheless, using this paradigm, we did detect connectivity differences at the same group-level random-effects threshold, suggesting that interactivity differences may be more robust than the activity differences observed in other studies. Moreover, these results demonstrate the importance of using multivariate methods in addition to univariate methods in order to fully investigate multidimensional data.

**Coherence as a Measure of Functional Connectivity**

We use coherence of fMRI data as an indicator of the functional interactivity between regions. Coherence is a spectral measure of the linear time-invariant relationship between 2 time series and has been applied to functional neuroimaging data such as EEG, magnetoencephalography, and single-cell electrophysiology (Rappelsberger and others 1988; Rosenberg and others 1989; Schack and others 1999). Whereas these methods typically use coherence of a large range of frequencies (10–150 Hz), the bandwidth of the fMRI BOLD signal is limited by the hemodynamic response. Therefore, we monitored the coherence of a low-frequency band (0–0.15 Hz).

Low-frequency connectivity has been investigated in fMRI data, primarily using correlation. Several studies have reported low-frequency correlations between homologous primary motor, auditory, and visual cortices at rest (Biswal and others 1995; Xiong and others 1999; Cordes and others 2000; Lowe and others 2000), as well as between relevant ROIs during the performance of a task (Hampson and others 2002). These correlations are supported by our current understanding of anatomical and functional connectivity. Additionally, the study by Quigley and others (2003) showed that patients with agenesis of the corpus callosum had diminished correlations between homologous primary sensory and motor regions compared with normal healthy subjects. This provides further evidence that low-frequency fluctuations can be used as an indicator of connectivity. Coherence has an important advantage over correlation in that it is invariant to interregional differences in the HRF. However, like all symmetric bivariate measures (correlation included), coherence is insensitive to directionality.

**Conclusion**

In this study, we use coherence of fMRI data to investigate changes in functional connectivity of the motor network during bimanual sequence learning. To our knowledge, this is the first fMRI study using coherence to investigate dynamic learning-related changes of interregional coupling. As with previous connectivity studies in EEG, our results support a functional role for the enhanced interhemispheric coupling within the motor network during the early stage of learning. Additionally, our results highlight a coupling between the frontal network and the motor network during early learning. These results provide an integrative and dynamic view of the cortical network during motor skill learning.

**Notes**

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