

# Learning from the other limb's experience: sharing the 'trained' M1 representation of the motor sequence knowledge

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## Key points

- Participants were scanned during the untrained-hand performance of a motor sequence, intensively trained a day earlier, and also a similarly constructed but novel, untrained sequence.
- The superior performance levels for the trained, compared to the untrained sequence, were associated with a greater magnitude of activity within the primary motor cortex (M1), bilaterally, for the trained sequence.
- The differential responses in the 'trained' M1, ipsilateral to the untrained hand, were positively correlated with experience-related differences in the functional connectivity between the 'trained' M1 and (1) its homologue and (2) the dorsal premotor cortex (PMd) within the contralateral hemisphere.
- No significant correlation was evident between experience-related differences in M1 – M1 and M1 – PMd connectivity measures.
- These results suggest that the transfer of sequence-specific information between the two primary motor cortices is predominantly mediated by excitatory mechanisms driven by the 'trained' M1 via two independent neural pathways.

**Abstract** Following unimanual training on a novel sequence of movements, sequence-specific performance may improve overnight not only in the trained hand, but also in the hand afforded no actual physical experience. It is not clear, however, how transfer to the untrained hand is achieved. In the present study, we examined whether and how interaction between the two primary motor cortices contributes to the performance of a sequence of movements, extensively trained the day before, by the untrained hand. Accordingly, we studied participants during the untrained-hand performance of a finger-to-thumb opposition sequence (FOS), intensively trained a day earlier (T-FOS), and a similarly constructed, but novel, untrained FOS (U-FOS). Changes in neural signals driven by task performance were assessed using functional magnetic resonance imaging. To minimize potential differences as a result of the rate of sequence execution *per se*, participants performed both sequences at an identical paced rate. The analyses showed that the superior fluency in executing the T-FOS compared to the U-FOS was associated with higher activity within the primary motor cortex (M1), bilaterally, for the T-FOS. The differential responses in the 'trained' M1 were positively correlated with experience-related differences in the functional connectivity between the 'trained' M1 and (1) its left homologue and (2) the left dorsal premotor cortex. However, no significant correlation was evident between the changes in connectivity in these two routes. These results suggest that the transfer of sequence-specific information between the two primary motor cortices is predominantly mediated by excitatory mechanisms driven by the 'trained' M1 via at least two independent neural pathways.

(Received 14 January 2015; accepted after revision 1 October 2015; first published online 7 October 2015)

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**Abbreviations** AAL, automated anatomical labelling; BOLD, blood oxygen level-dependent; CC, correlation coefficient; CV, contrast value; fMRI, functional magnetic resonance imaging; FOS, finger-to-thumb opposition sequence; FWE, family-wise error; HMAT, human motor area template; L, left; M1, primary motor cortex; MNI, Montreal Neurological Institute; PMd, dorsal premotor cortex; R, right; RE, repetition enhancement; ROI, region of interest; RS, repetition suppression; SMA, supplementary motor area; T-FOS, trained finger-to-thumb opposition sequence; TMS, transcranial magnetic stimulation; U-FOS, untrained finger-to-thumb opposition sequence.

## Introduction

Motor practice often results in improved performance but in a task-specific manner. However, some training-related gains can be generalized (i.e. transferred) to novel conditions, novel contexts and untrained motor effectors (Cohen *et al.* 1990; Grafton *et al.* 1998, 2002; Japikse *et al.* 2003; Korman *et al.* 2003; Perez *et al.* 2007*b*). The generalization of acquired knowledge presumably reflects the way in which the memory is encoded and consolidated and thus provides some insights into the nature and level of the task representation changes that occur during different phases of skill learning (Adams, 1987; Karni, 1996; Censor, 2013). Following unimanual practice, the nature of the internal representations subserving the training-related gains in performance can be assessed by testing for generalization to the contralateral, untrained hand. Studies indicate that an effective sequence-specific (i.e. preserving the same order of component movements) but effector independent representation of a trained movement sequence can develop early in practice (Grafton *et al.* 2002; Korman *et al.* 2003; Perez *et al.* 2007*b*, 2007*a*; Panzer *et al.* 2009; Amemiya *et al.* 2010). This ability to implement knowledge of the movement sequence by the untrained hand was conceptualized as reflecting experience-driven changes in an 'intrinsic' movement-based, encoding system (Willingham, 1998; Hikosaka *et al.* 1999) and implicates an interaction between the primary motor cortices (M1) in sequence representation (Grafton *et al.* 2002; Romei *et al.* 2009). However, the neural mechanisms underlying the ability to transfer training-related gains, for a practised movement sequence, to the contralateral, untrained, hand remain poorly understood.

Unilateral hand movements are predominantly controlled via direct pathways to the spinal cord descending from the contralateral M1 (Brinkman & Kuypers, 1973). Thus, unimanual practice would be assumed to affect predominantly the M1 contralateral to the trained hand. However, there is evidence for changes in neural activity within the ipsilateral M1 (Kim *et al.* 1993; Kobayashi *et al.* 2003; Verstynen *et al.* 2005; Verstynen & Ivry, 2011). Consequently, unilateral training may lead to practice-related changes in both, the contralateral

('trained') and the ipsilateral ('untrained') primary motor cortices. Such changes in the organization of the 'untrained' M1, ipsilateral to the trained hand, may subsequently contribute to the improved performance of the untrained hand, as suggested, for example, in a study of ballistic finger movements (Lee *et al.* 2010). The 'untrained' M1 may also be implicated in an effector independent representation of movement sequences (Perez *et al.* 2007*a*; Romei *et al.* 2009).

Assuming that learning-related changes affect an effector-dependent representation of movement in the 'trained' M1, the transfer of training-related gains to the untrained hand may occur via ipsilateral uncrossed descending projections (Ziemann *et al.* 1999). However, evidence for direct corticospinal contributions to the control of the ipsilateral distal upper limb in mammals is lacking (Lassonde *et al.* 1995; Kobayashi *et al.* 2003; Soteropoulos *et al.* 2011; Zaaïmi *et al.* 2012). Alternatively, the 'trained' M1 may contribute to intermanual transfer via transcallosal connections between the two hemispheres (Kobayashi *et al.* 2003; Davare *et al.* 2007), thus 'educating' the 'untrained' M1 when the trained motor sequence is performed with the untrained hand. However, the transcallosal connections between the primary motor cortices are mainly considered to be inhibitory (Ferber *et al.* 1992; Meyer *et al.* 1995; Hanajima *et al.* 2001) and, presumably, would limit intermanual transfer, rather than contribute to it. Supportive evidence for this notion come from studies showing that 'virtual lesion' to M1, by applying repetitive transcranial magnetic stimulation (TMS), may lead to improved performance in the ipsilateral hand, presumably as a result of the suppression of transcallosal inhibition (Gorsler *et al.* 2003; Kobayashi *et al.* 2004; Romei *et al.* 2009).

Unimanual practice may lead to reduced inter-hemispheric inhibition between the primary motor cortices as well (Shim *et al.* 2005; Perez *et al.* 2007*b*; Camus *et al.* 2009). Thus, practice-driven changes in excitatory-inhibitory balance between motor cortices may support the generalization of the training-related gains in motor performance to the untrained hand (Perez *et al.* 2007*b*; Camus *et al.* 2009). However, the amount of interhemispheric inhibition after training was found to correlate with non-specific improvement

of performance by the untrained hand, and not with movement-based sequence-specific intermanual transfer (Perez *et al.* 2007b).

The effector independent representation of the trained movement sequence may nevertheless depend on the recruitment of motor regions hierarchically higher than M1 (Hikosaka *et al.* 1999, 2002). Thus, the interactions between the 'untrained' M1 and high-level associative cortical regions such as the supplementary motor area (SMA) and the dorsal premotor cortex (PMd) may facilitate the performance of a trained movement sequence by the untrained hand (Grafton *et al.* 2002; Bischoff-Grethe *et al.* 2004; Perez *et al.* 2007b, 2007a, 2008; Wiestler *et al.* 2014).

The intermanual transfer of performance gains acquired in unimanual training on a sequence of movements (thus involving homologous fingers to those used during the training) was reported following a single session of training (Grafton *et al.* 2002; Korman *et al.* 2003; Kirsch & Hoffmann, 2010; Amemiya *et al.* 2010; but see also Witt *et al.* 2010). Importantly, following a single session of practice on an explicitly introduced finger-to-thumb opposition sequence (FOS), using the left, non-dominant hand, sequence-specific performance improved overnight in both the trained and the untrained hand (Korman *et al.* 2003).

In the present study, using functional magnetic resonance imaging (fMRI), we tested which motor areas showed a differential pattern of activity and connectivity when a set of finger movements was executed in a well-trained and consolidated sequence (movement order) compared to the same movements arranged in a previously unpractised order using the untrained (right) hand. Noteworthy, experience with the trained movement sequence was provided only to the left hand and, before the brain scanning session, no actual physical experience of the specific sequence was afforded to the right hand. Thus, we compared changes in neural signals evoked during the performance of two sequences, trained and untrained (T-FOS and U-FOS, respectively), composed of the same component movements and performed at an identical, paced rate. We have shown previously that the level of experience with a specific movement sequence is reflected in the pattern and magnitude of short-term brain activity modulations upon task repetition when executed with the trained hand (Karni *et al.* 1995; Gabitov *et al.* 2014, 2015). We therefore also tested whether previous experience with a motor sequence in one hand is reflected in repetition-driven modulations of the neural activity when performed with the untrained hand. Specifically, we examined whether and how the interaction between the two primary motor cortices contributes to the effector independent representation of a trained sequence of movements. In addition, the possible contributions of the higher-level associative

premotor regions, SMA and PMd, were explored. The results of the present study suggest that the motor sequence representation within the 'trained' M1 may also contribute to the performance of the task using the untrained hand. This sequence-specific intermanual transfer cannot be exclusively explained by direct transcallosal connections between the two primary motor cortices.

## Methods

### Participants

Thirty-two healthy young adults participated in the present study for payment: 17 participants (range 19–35 years; mean  $\pm$  SD, 25.7  $\pm$  4.4 years; five females) in the fMRI group and 15 participants (range 20–35 years; mean  $\pm$  SD, 25.47  $\pm$  2.73 years, eight females) in the control group. Both groups were trained and behaviourally tested in an identical protocol, whereas only participants of the fMRI group underwent the imaging session. Thus, the control group was tested to evaluate the possible effects on subsequent performance of the additional experience afforded during the fMRI session. Two participants from the fMRI group were not included in the analysis: one had difficulties with executing the task in the scanner; another withdrew from the fMRI session for personal reasons. All participants reported no prior history of neurological or psychiatric illness or brain injury and no addiction to drugs, alcohol or cigarettes (non-smokers or occasional smokers). Exclusion criteria included current or chronic use of medication, any known learning disabilities and attention deficit disorder. Only individuals with little (<2 years) or no formal music training participated in the present study. Professional typists were excluded as well. All participants affirmed that they had no sleep disorders and reported at least 6 h of proper night sleep during the study period. Each participant was identified as being strongly right handed using the Edinburgh Handedness Inventory (Oldfield, 1971). Prior to the study, all participants provided their written informed consent according to a protocol approved by the Ethics Committee of the C. Sheba Medical Centre.

### Design and procedures

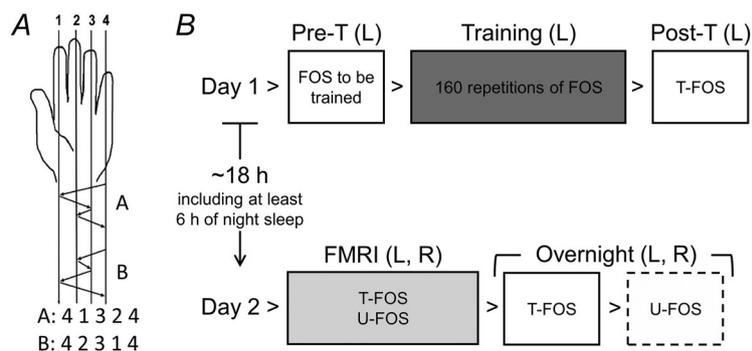
Participants were trained to accurately perform a given five-element finger-to-thumb opposition sequence, either sequence A or sequence B, with their non-dominant left hand (Fig. 1A). Both sequences consisted of identical component movements and were mirror-reversed in relation to each other. Thus, the two sequences were matched for the number of movements per digit and differed only in their order. If the sequence assigned for training was A (T-FOS), then sequence B was used as the

novel untrained sequence (U-FOS) and vice versa. The movement sequence was randomly assigned and explicitly instructed. In all sessions and tests, the participants performed the instructed movement sequence lying supine. The executing hand was positioned beside the trunk in direct view (palm-up) of a video camera to allow the recording of all digit movements. Visual feedback was not afforded at any time.

Each participant took part in two study sessions conducted on two consecutive days, separated by an 18 h interval that included nocturnal sleep (Fig. 1B). On day 1, all participants were trained and tested according to a standard FOS training protocol (Korman *et al.* 2003, 2007); for details, see our previous report (Gabbitov *et al.* 2014). On the second day, all participants were retested on the performance of the trained sequence and then the untrained sequence (overnight: T-FOS and U-FOS, respectively) using the untrained (right) as well as the trained (left) hand. The results for the trained hand have been reported previously (Gabbitov *et al.* 2014, 2015). The performance test for each condition included four consecutive blocks of 30 s in duration separated by rest intervals of 30 s. Before each test-block, participants were asked to perform the movement sequence, and the block was initiated only after the FOS was accurately reproduced three times in a row. Each test-block was initiated and terminated by an auditory 'READY' and 'STOP' signal, respectively. Participants were instructed to perform the sequence continuously 'as fast and as accurately as possible' and, in case of an error being noted, not to correct errors but rather to continue from the initial movement of the assigned sequence as smoothly as possible. No feedback on performance was provided. The performance of each participant during the test-blocks

was recorded by a video camera and scored offline. For each test-block, two measures of performance were determined from these recordings: (1) the number of correctly completed sequences as a measure of speed and (2) the number of incorrect sequences (errors) as a measure of accuracy.

Prior to the overnight performance tests, participants of the fMRI group took part in a scanning session, wherein they were asked to perform either the sequence trained the day before (T-FOS) or the novel sequence (U-FOS), using their untrained (right) hand. The trained (left) hand was tested as well; the results are reported elsewhere (Gabbitov *et al.* 2014, 2015). The imaging session consisted of three consecutive runs for each sequence. In this way, potential effects of proactive interference and contextual retrieval that could be caused by switching between the two sequences were minimized (Cothros *et al.* 2006; Kiesel *et al.* 2010). The order of sequences was counterbalanced across participants. Experimental runs (each 144 s long) were separated by a 1.5–2 min break, which included a verbal interaction with the participant. The component movements of the sequences were paced by an auditory signal at a fixed rate of 1.66 Hz to control rate-related changes in the blood oxygen level-dependent (BOLD) signal (Rao *et al.* 1996). The paced performance enabled the assessment of signal differences as a function of the order of the component movements minimizing potential differences between the T-FOS and U-FOS, which were expected to result from training on one but not the other sequence (Karni *et al.* 1995; Korman *et al.* 2003), as well as minimizing differences in performance rates between individuals. Each imaging run was initiated only after the explicitly designated FOS was accurately reproduced three times in a row. The run consisted of two



**Figure 1. Study design**

A, finger-to-thumb opposition sequences (FOSes). The two sequences were matched for number of movements per digit and arranged in the reversed order in relation to each other. B, overall study design. L, left hand; R, right hand. Day 1: a pre-training performance test (Pre-T), a structured training session (Training) and an immediate post-training performance test (Post-T) using the left hand (L). Day 2, performance tests of the trained sequence and the untrained sequence (overnight: T-FOS and U-FOS, respectively) using the left, trained and the right, untrained hand (L and R, respectively). Only participants of the fMRI group took part in the scanning session (fMRI), immediately preceding overnight performance tests. The control (non-fMRI) group was tested to evaluate the effect of the additional experience afforded during the fMRI session on subsequent performance.

performance-blocks (Block1 and Block2) separated by a rest interval of 30 s (Fig. 2A). Each block was initiated by an auditory and visual 'READY' cue (2 s), after which participants performed the required FOS continuously in a paced manner for a total of eight repetitions of the FOS (24 s). The end of the performance-block was marked by an auditory and visual 'STOP' cue (1 s).

The performance of participants during the fMRI session was recorded by a video camera focused on the performing hand, and evaluated by at least one trained observer, both online and offline. Performance was evaluated for accuracy, timing (i.e. initiation and termination of FOS performance) and performance rate to ensure an appropriate task execution. Errors occurred very rarely and, when they were noted by the experimenters or the participants, the run was repeated. No additional errors were observed during evaluation of performance offline. Only runs with errorless performance were included in the analyses. This experiment was realized using Cogent 2000, developed by the Cogent 2000 team at the Functional Imaging Laboratory and the Institute of Cognitive Neuroscience (University College London, London, UK), and Cogent Graphics, developed by John Romaya at the Laboratory of Neurobiology (Wellcome Department of Imaging Neuroscience, London, UK), and implemented in MATLAB (The Mathworks Inc., Natick, MA, USA).

### fMRI data acquisition

fMRI scanning was carried out at the C. Sheba Medical Centre, Tel-Hashomer, using a 3 Tesla whole body high definition system (EXCITE 3 HD; GE Healthcare Ltd, Little Chalfont, UK) equipped with an eight-channel head coil. A high-resolution full-brain 3-D structural images were acquired in the axial orientation using a T1\*-weighted echo-planar sequence (TR = 7.3 ms, TE = 3 ms, flip angle = 20°, FOV = 256 × 256 mm<sup>2</sup>, matrix size = 256 × 256 voxels, voxel size = 1 × 1 × 1 mm<sup>3</sup>). BOLD-sensitive functional images were obtained using a gradient echo-planar T2\*-sequence (TR = 3000 ms, TE = 35 ms, flip angle = 90°, FOV = 220 × 220 mm<sup>2</sup>, matrix size = 64 × 64 voxels, voxel size = 3.4 × 3.4 × 3.4 mm<sup>3</sup>, no gap, ascending) with 40 axial oblique slices, covering the whole brain.

### fMRI analysis

**Preprocessing.** The structural and functional images were converted to the Neuroimaging Informatics Technology Initiative (NIFTI) format using MRICron (University of South Carolina). Preprocessing and statistical analysis of the data were carried out with Statistical Parametric

Mapping (SPM8) (Wellcome Department of Cognitive Neurology, London, UK) operating under Matlab R2012a (The Mathworks Inc.). For each run, the four initial scans were discarded to allow for magnetic saturation and equilibration effects. First, all images were re-orientated to stereotactic space. All functional volumes were realigned using a least squares approach and a six parameter (rigid body) spatial transformation to remove movement-related variance. To correct for non-rigid distortion, realigned functional volumes were unwarped, adjusting for interactions between movement and local field inhomogeneity (Andersson *et al.* 2001; Hutton *et al.* 2013). This dynamic geometric distortion correction reduces motion-related variance and improves the temporal signal-to-noise ratio (Andersson *et al.* 2001; Hutton *et al.* 2013). Following segmentation and skull-stripping of the structural data, functional images were co-registered to the individual skull-stripped 3-D anatomical image and normalized to the Montreal Neurological Institute (MNI) space using parameters obtained from the segmentation procedure. The normalized functional images were resampled to voxel dimensions of 3 mm<sup>3</sup>. Finally, functional images were spatially smoothed with a Gaussian kernel of 8 mm full width at half-maximum to improve the signal-to-noise ratio. Prior to statistical analyses, head motion artefact detection routine was applied on the preprocessed data using artefact detection tools (Mazaika *et al.* 2009). No significant head motion artefacts were detected (normalized z-threshold = 2, movement threshold = 2 mm, rotation threshold = 0.05 rad).

**Statistical parametric models.** Statistical analyses of BOLD signal changes were performed using a general linear model (Friston *et al.* 1995). Individual models were specified separately for each condition (T-FOS and U-FOS performed with either the trained (left, L) or the untrained (right, R) hand) using a multisession design, whereas each session included data from a single run (three runs). Regressors of interest (i.e. Block1 and Block2) were modelled as a boxcar function with a length of 24 s convolved with the canonical haemodynamic response function. A high pass filter of 128 s was used to remove low-frequency noise. Following the model parameters estimation, the linear contrasts for each *sequence* (T-FOS and U-FOS) were defined as: Perf *vs.* Rest [i.e. (Block1 + Block2) *vs.* Rest] to assess task-related changes in the BOLD-fMRI signal; and Block1 *vs.* Block2 to assess changes in the BOLD-fMRI signal upon task repetition following the brief rest interval (i.e. across blocks).

**Whole-brain analysis.** To investigate group effects, contrast images of each effect of interest (Perf *vs.* Rest

**Table 1. Areas of interest used for small volume corrections**

<i>n</i>	Area of interest	Image calculation
1	Right primary sensorimotor cortex	S1 (R) + M1 (R)
2	Left primary sensorimotor cortex	S1 (L) + M1 (L)
3	Supplementary motor area	SMA proper (L + R) + pre-SMA (L + R)
4	Right dorsal lateral premotor cortex	PMd (R)
5	Left dorsal lateral premotor cortex	PMd (L)
6	Right ventral lateral premotor cortex	PMv (R)
7	Left ventral lateral premotor cortex	PMv (L)
8	Right medial temporal lobe	Hippocampus (R) + ParaHippocampal (R)
9	Left medial temporal lobe	Hippocampus (L) + ParaHippocampal (L)

+, union; R, right hemisphere; L, left hemisphere; S1, primary sensory cortex (HMAT); M1, primary motor cortex (HMAT); SMA proper, supplementary motor area proper (HMAT); pre-SMA, pre-supplementary motor area (HMAT); PMd, dorsal lateral premotor cortex (HMAT); PMv, ventral lateral premotor cortex (HMAT); hippocampus (AAL); parahippocampal, parahippocampal gyrus (AAL).

and Block1 vs. Block2) were introduced into second-level analyses separately for each *sequence* treating participants as a random effect (one-sample *t* test). Differential activation as a function of a *sequence* (T-FOS and U-FOS) performed with the untrained hand was assessed using a one-way within-subject repeated measures ANOVA design. Activation maps were thresholded at  $P \leq 0.001$  uncorrected and overlaid on the mean structural image of all participants using SPM8 and the Functional Imaging Visualization Environment (<http://nmr.mgh.harvard.edu/harvardagingbrain/People/AaronSchultz/OrthoView.html>). Statistical inferences were performed on the cluster-level using *P* values family-wise error (FWE) rate corrected for multiple comparisons over the entire brain or on the peak-level using *P* values FWE-corrected over a small volume of interest. Areas of interest for small volume corrections were defined for structures within the cortical–subcortical network implicated in motor learning (Halsband & Lange, 2006; Hardwick *et al.* 2013). Primary sensory and motor cortex, dorsal premotor cortex and SMA (pre-SMA and SMA-proper) were determined using the human motor area template (HMAT) (Mayka *et al.* 2006). Subcortical structures were determined using automated anatomical labelling (AAL) (Tzourio-Mazoyer *et al.* 2002). Areas of interest relevant to the reported statistics are listed in Table 1.

**Region of interest (ROI) definition.** According to our *a priori* purpose, ROIs within the primary motor cortices were defined using the central sulcus and the hand knob (Yousry *et al.* 1997) contralateral to the performing hand as an anatomical mark. Additional ROIs within premotor areas were localized within locations and boundaries of SMA (pre-SMA and SMA-proper) and PMd, as defined in the HMAT. The functional voxels relevant to the task were identified on an individual level from activation maps of the increased BOLD-fMRI signal during performance-blocks (Perf > Rest) for each *task*

(T-FOS and U-FOS) and each hand (right and left) using family-wise error correction at  $P < 0.05$ . The present study refers to analyses of data acquired during the untrained (right) hand performance. Data acquired during the trained (left) hand performance was used only for ROI definitions. The MNI co-ordinates of the most active voxel (local maxima) within M1 hand areas contralateral to the performing hand (i.e. the ‘untrained’ and ‘trained’ M1, predominantly activated during the right-hand’s and left-hand’s performance, respectively), as well as SMA and PMd, bilaterally, were extracted from individual activation maps and averaged across conditions accordingly. Finally, six group ROIs for M1, SMA and PMd, bilaterally, were defined as spheres with a radius of 6 mm, centred at MNI co-ordinates averaged across participants for each functional region.

**ROI analysis.** ROI analyses were performed to assess differences in neural activity as a function of prior experience within M1, using the MarsBar toolbox for SPM (Brett *et al.* 2002). For each ROI within the ‘untrained’ and ‘trained’ M1, betas and contrast values for performance-related increases in the BOLD-fMRI signal (CV-Act: Perf > Rest) and repetition effects across blocks [CV-RE: Block1 < Block2; positive and negative values correspond to relative increases, i.e. repetition enhancement (RE), and relative decreases, i.e. repetition suppression (RS) in the BOLD-fMRI signal across blocks, respectively] were extracted for each *sequence* (T-FOS and U-FOS).

To explore possible differences between sequences in repetition effects within blocks (e.g. Gabbitov *et al.* 2015), raw time-courses for each ROI were extracted from pre-processed functional images for each run. These raw BOLD signals were converted to percentage signal change. To reduce the low-frequency noise as a result of scanner drift, the BOLD signal at the performance-block’s onset (‘READY’ cue) was used as the block’s baseline. Each

block was divided into two equal phases (Phase1 and Phase2), each consisting of four successive time points (signal measurements), with Phase1, beginning 6 s after the 'READY' cue, and Phase2, including the 'STOP' cue; exclusion of time points corresponding to the first 6 s following the 'READY' cue minimized the effects of haemodynamic delay.

The analyses were designed as within-subject comparisons. ANOVA or paired samples *t* tests were run using SPSS, version 19.0 (IBM Corp., Armonk, NY, USA). The results were corrected for non-sphericity violation using the Greenhouse–Geisser adjustment. BOLD signals within SMA and PMd were also assessed using similar approach.

**Functional connectivity analysis.** To explore interactions between M1, SMA and PMd during the untrained (right) hand performance, ROI-to-ROI functional correlations were assessed using the Functional Connectivity Toolbox (Conn) for SPM (Whitfield-Gabrieli & Nieto-Castanon, 2012). Prior to estimation of connectivity indices, the pre-processed functional data underwent additional temporal preprocessing. Six parameters obtained by rigid body head motion correction (three-rotation and three-translation parameters), plus six additional parameters representing the corresponding first-derivative terms, were used as temporal covariates aiming to reduce the impact of motion within performance-blocks. Between-blocks main effects may affect within-block connectivity estimates in the presence of possible voxel-specific differences in haemodynamic delay. Therefore, main effects of the performance-blocks (Block1 and Block2, each block 24 s long convolved with the canonical haemodynamic response function) and the corresponding first-derivative terms were included as additional temporal confounding factors. Temporal covariates were removed from the BOLD functional data using linear regression. The resulting residual BOLD time series were band-pass filtered ( $0.008 \text{ Hz} < f < 0.1 \text{ Hz}$ ) and divided into scans associated with each *block* (Block1 and Block2) separately for each *task* (T-FOS and U-FOS). To take into account the haemodynamic delay, block regressors were convolved with a canonical haemodynamic response function and rectified. Individual ROI-to-ROI connectivity matrices were computed for each *block* (Block1 and Block2) and each *task* (T-FOS and U-FOS) by estimating Pearson's correlation coefficients between ROIs. All ROI-to-ROI correlation coefficients were converted to normally distributed scores using Fisher's transformation to allow for second-level general linear model analyses. The connectivity patterns between ROIs were tested in second-level analyses for the main effect of *task* (T-FOS > U-FOS, T-FOS < U-FOS), the main effect of *block* (Block1 > Block2, Block1 < Block2) and *task* by *block* interactions. False positive control

in ROI-to-ROI analyses was implemented using false discovery rate correction. Individual Fisher-transformed Pearson's correlation coefficients between the two functionally corresponding ROIs, a measure of inter-hemispheric connectivity, as well as between ROIs using M1 as a source were extracted and introduced into further correlation analyses.

**Correlation analysis.** Relationships between differences in BOLD signals and differences in functional connectivity, reflecting the previous experience with the sequence when performed with the untrained hand, were assessed in correlation analyses. The individual contrasts values for the mean performance-related activity (CV-Act: Perf > Rest) and mean Fisher-transformed correlation coefficients (CC, averaged across performance-blocks) were used for this purpose. For each individual, CV-Act during the U-FOS performance was subtracted from CV-Act during the T-FOS performance generating  $\Delta\text{CV-Act}$  values [ $\Delta\text{CV-Act} = (\text{CV-Act for the T-FOS}) - (\text{CV-Act for the U-FOS})$ ]. Differences in ROI-to-ROI functional connectivity between the two tasks were calculated in the same manner, generating  $\Delta\text{CC}$  values [ $\Delta\text{CC} = (\text{CC for the T-FOS}) - (\text{CC for the U-FOS})$ ].

### Behavioural data analysis

For each test-block performed by the untrained hand, two performance measures for each individual were determined: the number of correctly completed sequences as a measure of speed and the number of sequences with ordering errors as a measure of accuracy. In addition, the slope for speed as a measure of within-test improvement and the SD for speed as a measure of within-test variability were determined for each *sequence*. The slope was calculated as a gradient of linear regression line through four data points; each point represented speed achieved during one test-block. The SD for speed was converted to percents relative to individual mean speed achieved for each *sequence* performed with the untrained hand.

The analyses were designed as within-subjects comparisons. Separate repeated measures ANOVAs for each performance measure were run using SPSS. The results were corrected for non-sphericity violation using the Greenhouse–Geisser adjustment.

## Results

### fMRI results

During the fMRI session, participants were instructed to perform either the trained or the untrained sequence in a paced manner. Each run consisted of two

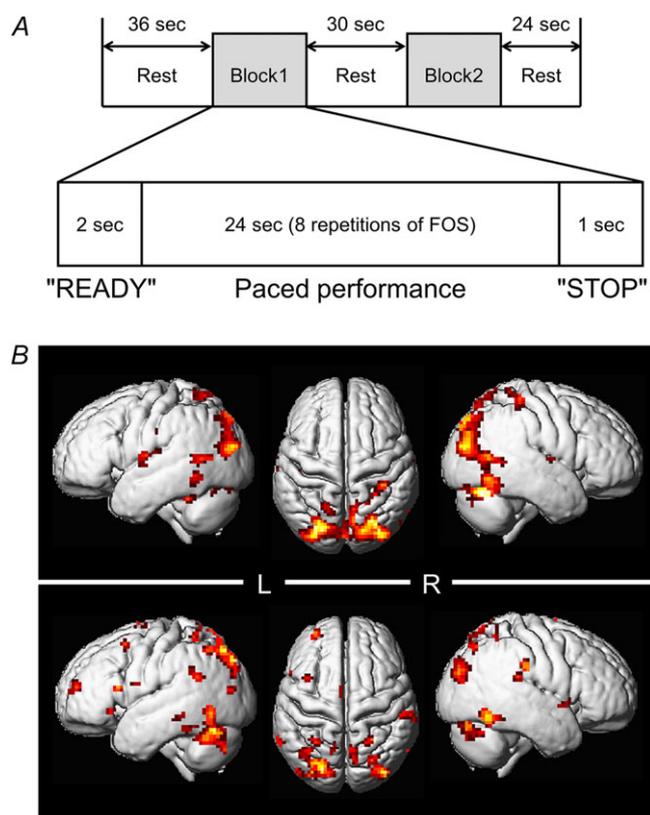
performance-blocks (Block1 and Block2) of the same sequence (T-FOS or U-FOS) separated by a brief rest interval (Fig. 2A). The present study refers to analyses of data acquired during the untrained (right) hand performance. Data acquired during task performance with the trained hand were used to define the ROIs.

**Whole-brain analysis.** Comparisons showed significantly greater BOLD signal for the trained sequence (T-FOS > U-FOS) only within a small cluster located in the left medial temporal lobe following small volume correction (Table 2, labelled as ParaHippocampal; AAL). There were no brain areas showing significantly greater activity for the untrained sequence (T-FOS < U-FOS). For both sequences, relative decreases in activity across performance-blocks (i.e. RS effects: Block1 > Block2) were

significant in multiple brain areas mainly located in posterior parts of the brain, the parietal and occipital lobes, as well as the cerebellum (Fig. 2B and Table 2). RS effects were also observed in dorsolateral and medial premotor areas, although the effects in these areas were significant only after small volume corrections. No significant increases in BOLD-fMRI signals across performance blocks (i.e. RE effects: Block1 < Block2) were observed for either *sequence* (T-FOS and U-FOS). There was no significant *sequence* by *block* interaction.

**Region of interest (ROI) analysis.** ROIs within the M1 hand areas (i.e. 'untrained' and 'trained' M1, predominantly activated during the right-hand's and left-hand's performance, respectively) were defined as spheres with a radius of 6 mm, centred at MNI co-ordinates averaged across participants (left M1:  $-38 \pm 0.49$ ,  $-24 \pm 1.05$ ,  $54 \pm 0.89$ ; right M1:  $40 \pm 0.41$ ,  $-19 \pm 0.82$ ,  $54 \pm 0.95$ , mean  $\pm$  SEM for  $x$ ,  $y$  and  $z$ , respectively) (Fig. 3A, middle).

To assess differences in neural signals within primary motor cortices as a function of previous experience with a *sequence* (T-FOS and U-FOS), performed with the untrained hand, contrast values extracted from statistical parametric models and BOLD signals during performance-blocks extracted from time-course series were analysed (Figs 3A and 4). Both analyses showed greater activity within the two primary motor cortices for the T-FOS compared to the U-FOS. A repeated measures ANOVA with *sequence* (T-FOS and U-FOS) and *hemisphere* (left and right) as within-subjects factors, performed on contrast values of performance-related increases in BOLD-fMRI signal (Perf > Rest) within the M1 ROIs, showed a significant effect of *sequence* ( $F_{1,14} = 4.54$ ,  $P = 0.05$ ) with no significant *sequence* by *hemisphere* interaction ( $F_{1,14} = 1.44$ ,  $P = 0.25$ ). In line with the predominant role of the contralateral hemisphere during unimanual action production (Brinkman & Kuypers, 1973), there was also significant effect of *hemisphere* ( $F_{1,14} = 89.55$ ,  $P < 0.001$ ). Thus, the activity during the performance-blocks was highly lateralized to the left M1, contralateral to the untrained hand. *Post hoc* paired sample  $t$  tests showed that differences in activity between the two sequences were significant only within the 'trained' M1, ipsilateral to the untrained hand ( $t_{14} = 1.79$ ,  $P = 0.10$ ;  $t_{14} = 2.14$ ,  $P = 0.05$ , left 'untrained' and right 'trained' M1, respectively). Analysis performed on contrast values of repetition effects across blocks (Block1 vs. Block2) showed no significant differences between the two sequences ( $F_{1,14} = 0.08$ ,  $P = 0.77$ ). However, an analysis of the signal intensity modulations across the three runs performed on the beta values showed a significant effect of *run* in the 'trained' M1 but only for the U-FOS ( $F_{1,88,26,27} = 3.56$ ,  $P < 0.05$ ); *post hoc* pairwise comparisons



**Figure 2. Design of a single fMRI run and results of the whole-brain analyses**

A, each run (144 s) consisted of two performance-blocks (Block1 and Block2) separated by a brief rest interval. The imaging session consisted of three consecutive runs for each sequence (T-FOS, U-FOS) separated by a 1.5–2 min break, which included a verbal interaction with the participant. B, activation maps showing RS effects across blocks for each task (T-FOS, upper panel; U-FOS, lower panel). Activation maps are shown over the surface rendered from the mean structural image of all subjects, thresholded at  $P < 0.001$  (uncorrected).

**Table 2. Whole-brain analysis**

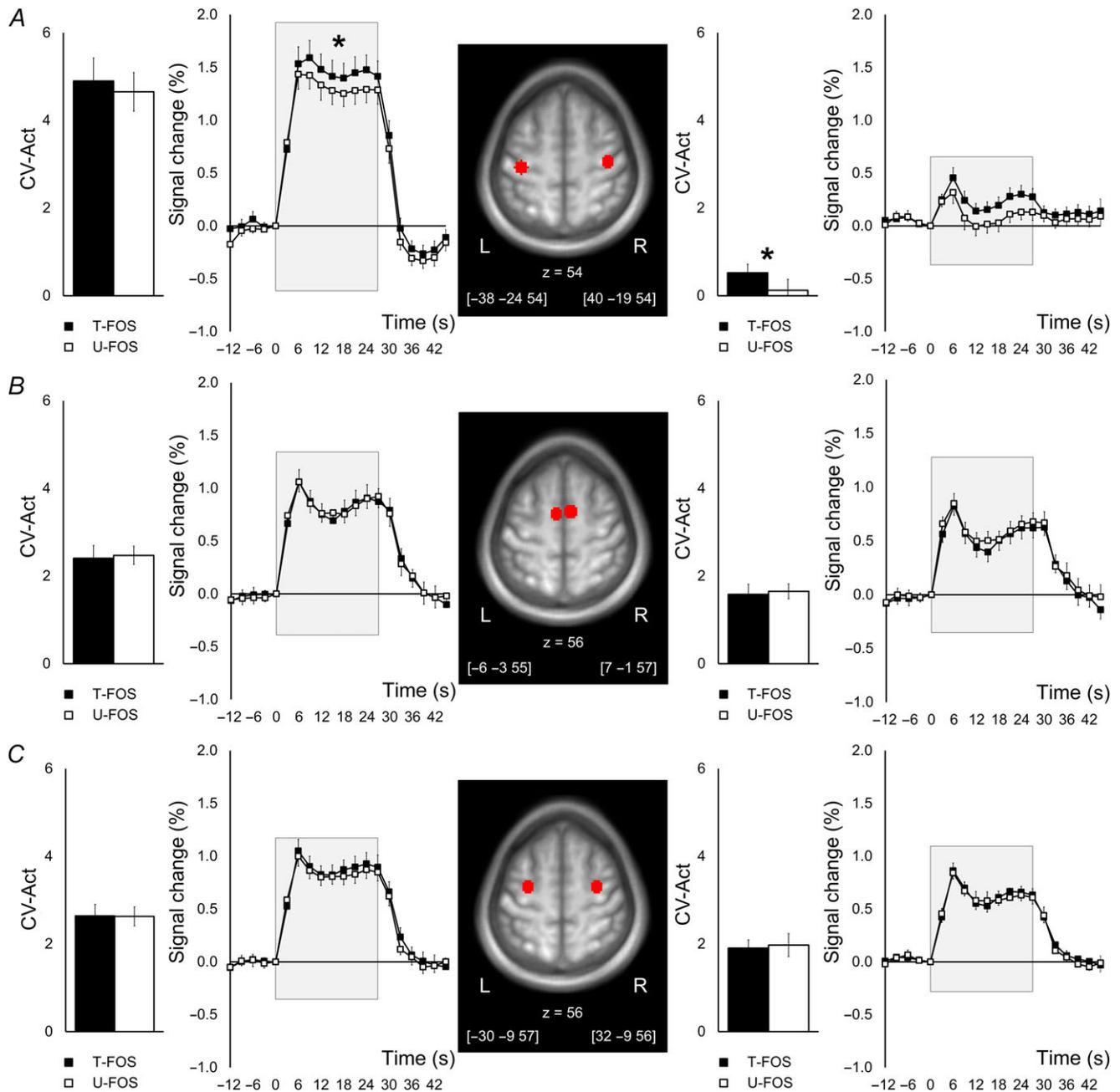
Label		MNI co-ordinates			Size (voxels)	z-score	p
		x	Y	z			
<i>T-FOS &gt; U-FOS</i>							
ParaHippocampal* <sup>9</sup>	L	-18	-31	-17	6	3.60	0.48
<i>RS for the T-FOS (Perf1 &gt; Perf2)</i>							
					1596		<0.001 <sub>FWE</sub>
Temporal_Inf	R	54	-61	-11		3.95	
Temporal_Mid	R	57	-67	7		3.93	
Cuneus	L	-6	-85	16		3.73	
Occipital_Sup	L	-21	-76	40		4.48	
Occipital_Mid	L	-36	-82	16		4.30	
Lingual	R	18	-82	-11		3.94	
Calcarine	L	-3	-73	16		3.84	
Lingual	L	-9	-76	-11		3.73	
Cerebellum_Crus1	R	54	-70	-26		4.17	
Cerebellum_Crus1	L	-6	-76	-26		3.95	
Cerebellum_6	R	18	-76	-17		4.08	
Cerebellum_6	L	-24	-67	-26		3.78	
					696		<0.001 <sub>FWE</sub>
Postcentral	R	12	-52	76		3.67	
Precuneus	R	6	-70	55		3.63	
Occipital_Sup	R	27	-70	40		4.32	
Occipital_Mid	R	30	-76	28		4.26	
					53		0.05
Postcentral* <sup>1</sup>	R	36	-34	61		4.33	
					17		0.26
Postcentral* <sup>1</sup>	L	-39	-16	34		3.74	
					37		0.1
Rolandic_Oper* <sup>7</sup>	L	-51	-4	10		3.62	
<i>RS for the U-FOS (Perf1 &gt; Perf2)</i>							
					252		<0.001 <sub>FWE</sub>
Parietal_Inf	L	-30	-76	49		4.09	
Occipital_Sup	L	-18	-82	43		4.82	
Occipital_Mid	L	-27	-82	37		4.29	
					1123		<0.001 <sub>FWE</sub>
Temporal_Mid	L	-45	-61	-5		3.79	
Occipital_Inf	L	-51	-64	-14		3.42	
Cerebellum_6	L	-15	-70	-26		4.73	
Cerebellum_6	R	15	-73	-29		4.31	
Cerebellum_8	L	-15	-67	-50		4.22	
Cerebellum_Crus1	R	39	-64	-29		3.25	
Cerebellum_Crus1	L	-6	-70	-29		4.45	
Cerebellum_Crus2	L	-21	-70	-38		4.02	
Cerebellum_Crus2	R	6	-70	-32		4.39	
					96		0.05 <sub>FWE</sub>
Temporal_Inf	R	57	-61	-11		3.73	
					14		0.26
Frontal_Mid* <sup>5</sup>	L	-36	11	55		3.58	
					15		0.25
Precentral* <sup>7</sup>	L	-39	2	19		3.95	
					22		0.17
Frontal_Inf_Tri* <sup>7</sup>	L	-48	14	16		3.50	

Labelling clusters (the most significant local maxima for each area) obtained from activation maps thresholded at  $P < 0.001$  (uncorrected) using AAL (Tzourio-Mazoyer *et al.* 2002). \*(<sup>n</sup>), significant peak at  $P < 0.05$  level FWE-corrected over a small volume of interest; (<sup>n</sup>) refers to an area of interest used for small volume correction as listed in Table 1;  $P_{FWE}$ , cluster-level FWE-corrected over the entire brain volume;  $P$ , cluster-level uncorrected.

showed significant decrease in signal intensity from the first to the second *run* ( $P = 0.01$ ) (Fig. 4B, middle).

The time-course series, for the two sequences, in terms of percentage signal change extracted from the M1 hand areas, are shown in Fig. 4 (right). A repeated

measures ANOVA with *sequence* (T-FOS and U-FOS), *hemisphere* (left and right), *block* (Block1 and Block2) and *phase* (Phase1 and Phase2) as within-subjects factors showed significant effect of *sequence* and *hemisphere* ( $F_{1,14} = 5.55$ ,  $P < 0.05$ ;  $F_{1,14} = 94.43$ ,  $P < 0.001$ ,



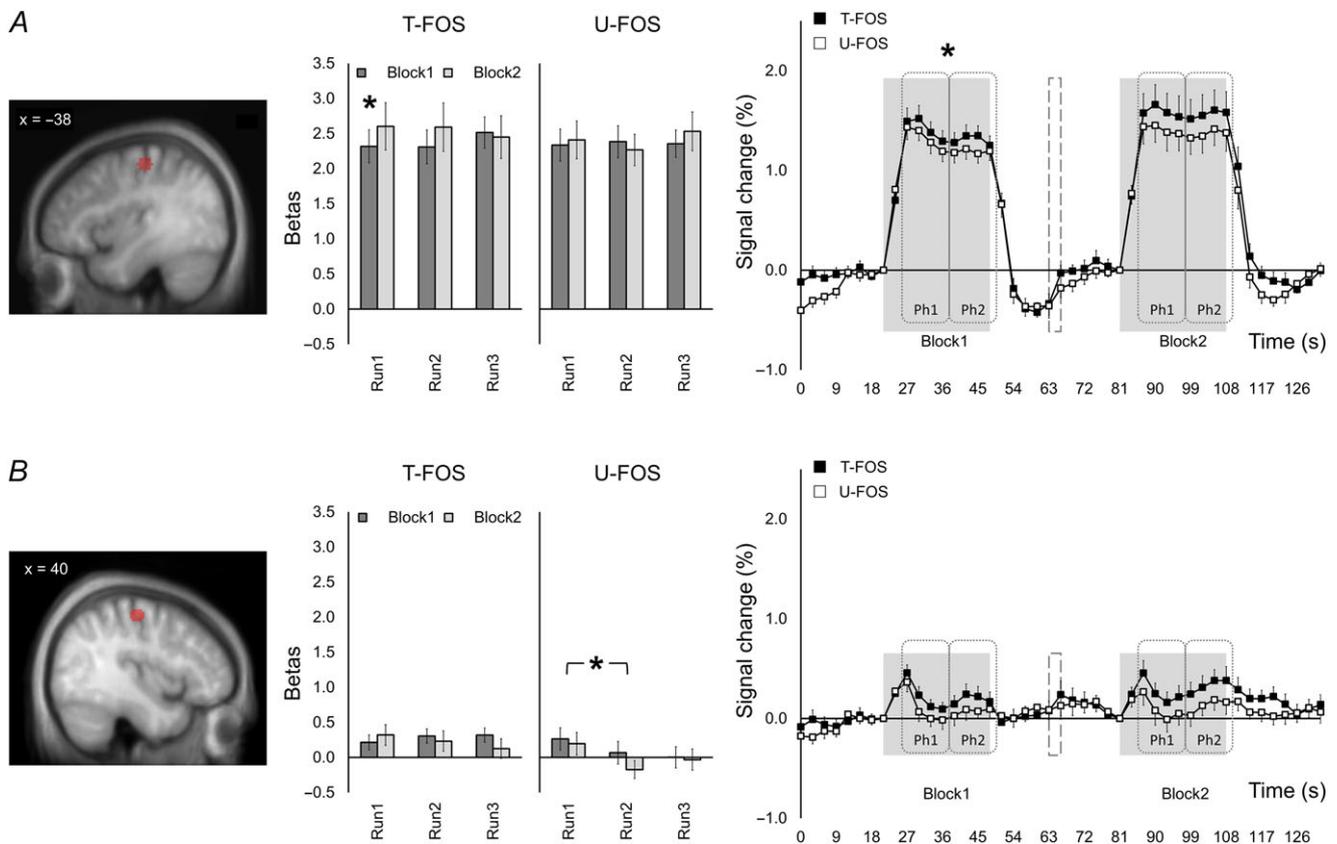
**Figure 3. Mean task-related activity and time-courses of mean signal intensity**

Group ROIs within M1, SMA and PMd, bilaterally (A, B and C, respectively) defined as spheres with a radius of 6 mm are shown over the mean structural image of all subjects (middle). Left hemisphere (L, left panels). Right hemisphere (R, right panels). Contrast values for performance-related increases in BOLD-fMRI signal (CV-Act: Perf > Rest) for each *sequence* (T-FOS and U-FOS) (left). Columns, CV-Act averaged across participants; bars, SEM. Mean time-courses in percentage signal change (vs. performance onset, 'READY' cue) across all sequence-specific runs averaged across performance-blocks are plotted vs. time (in seconds, counted from a performance onset, i.e. 'READY' cue = 0 s) (right). Data points, group mean percentage signal changes at a single time point; bars, SEM. \*Significant at  $P < 0.05$ .

respectively) with no significant *sequence* by *hemisphere* interaction ( $F_{1,14} = 0.02, P = 0.88$ ). *Post hoc* analyses, performed separately for each hemisphere, showed a trend towards greater activity within the ‘trained’ M1 during the T-FOS performance compared to the U-FOS ( $F_{1,14} = 3.76, P = 0.07$ ); the ‘untrained’ M1 showed significantly greater activity for the T-FOS ( $F_{1,14} = 6.42, P < 0.05$ ). There were neither significant modulations of the mean signal across blocks ( $F_{1,14} = 3.04, P = 0.10; F_{1,14} = 0.41, P = 0.53$ , main effect of *block* for the left ‘untrained’ and right ‘trained’ M1, respectively), nor significant *sequence* by *block* interactions ( $F_{1,14} = 0.69, P = 0.42; F_{1,14} = 0.22, P = 0.65$ , the left ‘untrained’ and right ‘trained’ M1, respectively). However, BOLD signals within the left M1 showed a significant *block* by *phase* interaction ( $F_{1,14} = 6.07, P < 0.05$ ), indicating that activity within the ‘untrained’ hemisphere varied between Phase1 and Phase2 as a function of task repetition across the brief rest interval; a similar trend was observed

within the right M1 ( $F_{1,14} = 3.57, P = 0.08$ , *block* by *phase* interaction).

For further exploration of the neural dynamics between phases and blocks, *post hoc* pairwise comparisons between phases were performed separately for each *hemisphere* and *sequence*. Comparisons of corresponding phases across blocks did not show any significant differences. However, when the phases were compared within blocks, for both sequences, BOLD signals within the ‘untrained’ M1, contralateral to the performing hand, exhibited significant reductions from Phase1 to Phase2 within the first blocks of the pairs (i.e. within-block RS:  $P < 0.05; P = 0.001$ , T-FOS and U-FOS, respectively) but did not show modulations between the two phases within second, repeated blocks of the pairs ( $P = 0.52; P = 0.31$ , T-FOS and U-FOS, respectively). Thus, the ‘untrained’ M1 exhibited similar RS effects for both sequences, indicating that despite the previous experience with the T-FOS afforded a



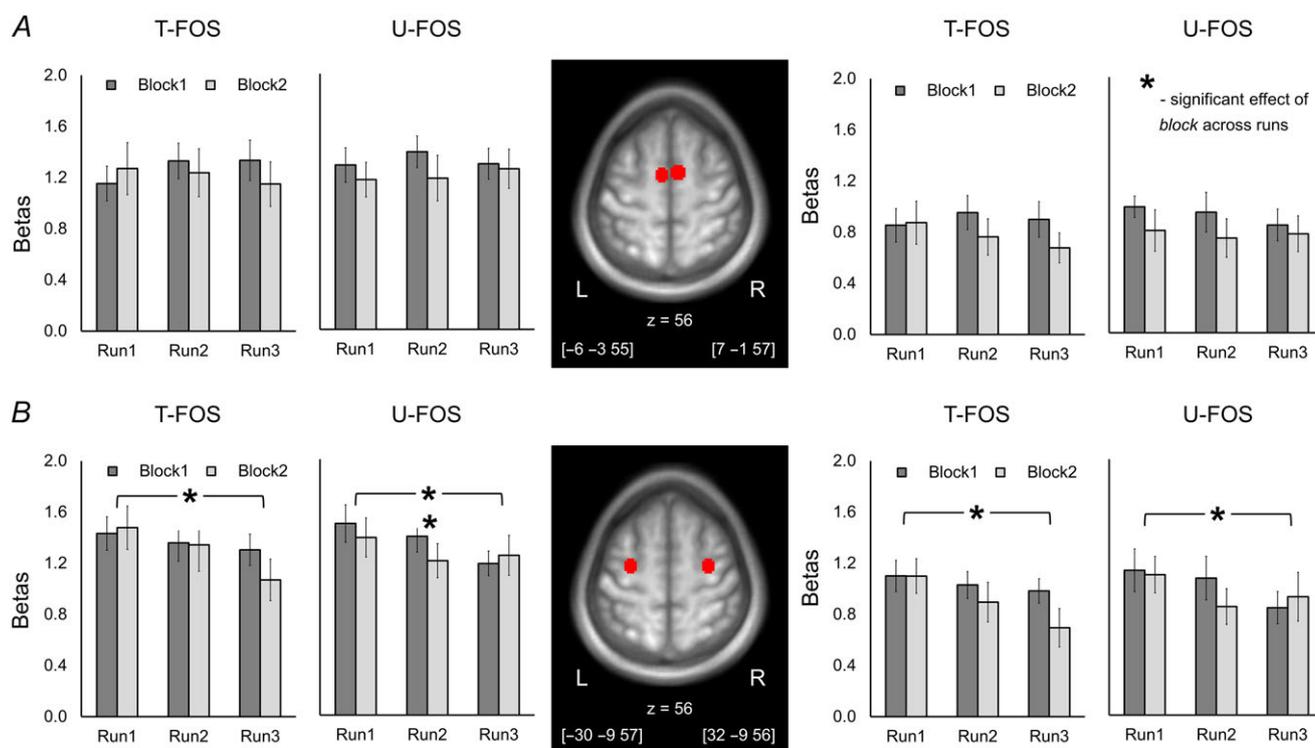
**Figure 4. Mean task-related activity and time-courses of mean signal intensity**  
 ROIs within the ‘untrained’ and ‘trained’ M1 centered at  $[-38, -24, 54]$  A, and  $[40, -19, 54]$  B, respectively. Sphered ROIs with a radius of 6mm are shown over the mean structural image of all subjects (left panels). Mean signals (betas) for each block (Block1 and Block2) and each run (1–3) are plotted separately for each sequence (T-FOS and U-FOS) (middle plots). Columns, betas averaged across participants; bars, SEM. Mean time-courses in percent signal change (versus performance onset, “READY” cue) across all sequence-specific runs are plotted versus time (in seconds) (right plots). Data-points, group mean percent signal changes at a single time-point; bars, SEM; Ph1 and Ph2, Phase1 and Phase2, respectively; dashed line, separation between time-points with BOLD signal normalized to the onset of Block1 and time-points with BOLD signal normalized to the onset of BLOCK2. \*Significant at  $P < 0.05$ .

day earlier to the left hand, there were no differential repetition-driven modulations of the neural activity for the T-FOS compared to the U-FOS, when performed with the untrained hand.

ROIs within the SMA and PMd, bilaterally, were defined as spheres with a radius of 6 mm, centred at MNI co-ordinates averaged across participants (left PMd:  $-30 \pm 1.63$ ,  $-9 \pm 1.15$ ,  $57 \pm 0.73$ ; right PMd:  $32 \pm 1.29$ ,  $-9 \pm 1.02$ ,  $56 \pm 1.47$ ; left SMA:  $-6 \pm 0.52$ ,  $-3 \pm 0.75$ ,  $55 \pm 1.39$ ; right SMA:  $7 \pm 0.78$ ,  $-1 \pm 1.43$ ,  $57 \pm 1.45$ , mean  $\pm$  SEM for  $x$ ,  $y$  and  $z$ , respectively) (Fig. 3B and C). Analyses of contrast values extracted from statistical parametric models, as well as analyses performed on the time-course series, showed no significant differences in the neural activity within premotor areas for the two sequences. A similar analysis, run on an additional ventral-anterior pre-SMA ROI centred at the local maxima within the pre-SMA extracted from the group activation maps of performance-related increases in activity (Perf > Rest;  $x = -6$ ,  $y = 8$ ,  $z = 46$ ), again showed no significant differences in the neural activity during the performance of the two sequences. Analyses of the beta values showed significant RS effects across blocks for the U-FOS within the right SMA ( $F_{1,14} = 4.86$ ,  $P < 0.05$ ) (Fig. 5A). Significant decreases in signal intensity across runs (from the first to

the third run) were observed within the PMd, bilaterally, for either *sequence* (Fig. 5B).

**Functional connectivity analysis.** Analyses of inter-hemispheric functional connectivity between ROIs within homologous cortical areas (M1 – M1, SMA – SMA and PMd – PMd) were performed on functional data acquired during the performance-blocks. None of these analyses showed any significant effect of *sequence* (T-FOS and U-FOS) performed with the untrained hand (Fig. 6). However, there was a significant across-blocks decrease (i.e. Block1 > Block2) in interhemispheric connectivity between the two primary motor cortices ( $t_{14} = 4.19$ ,  $P < 0.01$ ) with no *sequence* by *block* interaction. Nevertheless, analyses of *block* effects performed separately for each *sequence* showed a significant decrease in M1 – M1 interhemispheric connectivity across blocks only for the U-FOS ( $t_{14} = 1.41$ ,  $P < 0.67$ ;  $t_{14} = 3.01$ ,  $P < 0.05$ , T-FOS and U-FOS, respectively) (Fig. 6A). No significant changes in connectivity patterns across blocks were observed for ROIs within SMA and PMd with their homologue regions. Functional connectivity of each ROI within M1 with each ROI within premotor areas did not differ significantly between the two sequences.



**Figure 5. Betas extracted for ROIs within premotor areas**

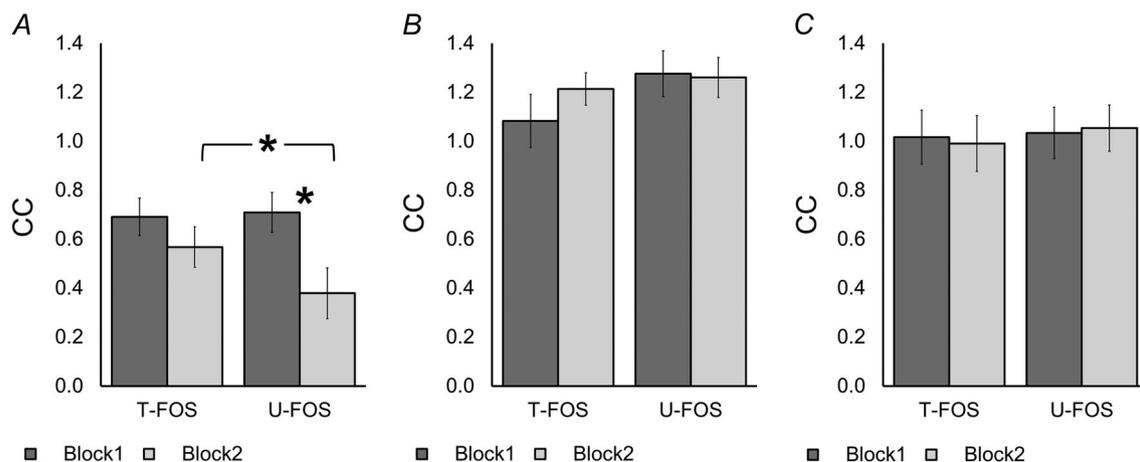
A, SMA. B, PMd. Left hemisphere (L, left panels). Right hemisphere (R, right panels). Mean signals (betas) for each *block* (Block1 and Block2) and each *run* (1–3) are plotted separately for each *sequence* (T-FOS and U-FOS). Columns, betas averaged across participants; bars, SEM. \*Significant at  $P < 0.05$ .

**Correlation analysis.** Relationships between the experience-related differences in the magnitude of activity ( $\Delta CV\text{-Act}$ ), exhibited by the primary motor cortices, and the experience-related differences in the strength of functional connectivity ( $\Delta CC$ ), using M1 as a source, were assessed in correlation analyses. Statistical inferences of the correlation analyses are listed in Table 3. Correlation analyses of  $\Delta CV\text{-Act}$  within the ‘untrained’ M1 and  $\Delta CC$  between the ‘untrained’ M1 and other ROIs did not show significant results (Table 3). Individual values of  $\Delta CV\text{-Act}$  within the ‘trained’ M1 were positively correlated with individual values of  $\Delta CC$  between the ‘trained’ M1 and its contralateral homologue (Fig. 7A, upper and Table 3).  $\Delta CV\text{-Act}$  within the ‘trained’ M1 was also positively correlated with  $\Delta CC$  between the ‘trained’ (right) M1 and the left PMd (Fig. 7A, lower and Table 3). However, *post hoc* correlation analysis of  $\Delta CC$  between the two primary motor cortices and  $\Delta CC$  between the ‘trained’ M1 and the left PMd did not show significant result ( $r = 0.35$ ,  $P = 0.62$ ) (Fig. 7B). Thus, the strength of the interhemispheric M1 – M1 connectivity and the strength of the right-M1 – left-PMd connectivity were modulated differentially by previous experience with a sequence.  $\Delta CC$  between the right M1 and the left PMd was positively correlated with  $\Delta CC$  between the left M1 and the left PMd ( $r = 0.67$ ,  $P < 0.05$ ) (Fig. 7C). These results suggest two independent neural pathways for the interhemispheric transfer of information between the two primary motor cortices, presumably driven by the ‘trained’ M1, in a sequence-specific manner.

### Behavioural results

The results of the behavioural performance test, using the untrained hand, undertaken after the imaging session

are shown in Fig. 8. The performance of the T-FOS was significantly faster, more accurate and less variable compared to the U-FOS. The T-FOS advantage was apparent irrespective of the experience with the two sequences inside the scanner as shown compared to a control group (participants who did not undergo an imaging session). A repeated measures ANOVA with *group* (fMRI, control) as a between-subjects factor as well as *sequence* (T-FOS and U-FOS) and *test-block* (1–4) as within-subjects factors showed a significant effect of *sequence* for both the number of correct sequences (i.e. speed) and the number of errors ( $F_{1,28} = 8.81$ ,  $P < 0.01$ ;  $F_{1,28} = 10.08$ ,  $P < 0.01$ , respectively). There was no significant effect of *group* ( $F_{1,28} = 0.64$ ,  $P = 0.43$ ;  $F_{1,28} = 3.78$ ,  $P = 0.062$ , the number of correct sequences and the number of errors, respectively) or *group* by *sequence* interaction ( $F_{1,28} = 0.07$ ,  $P = 0.79$ ;  $F_{1,28} = 0.50$ ,  $P = 0.49$ , the number of correct sequences and the number of errors, respectively). A trend towards a difference in accuracy of performance between the two groups was driven by the greater number of errors for the U-FOS in the fMRI group compared to controls ( $F_{1,28} = 1.50$ ,  $P = 0.23$ ;  $F_{1,28} = 5.45$ ,  $P < 0.05$ , T-FOS and U-FOS, respectively). Performance speed for both sequences improved rapidly during the test as indicated by a significant effect of *test-block* ( $F_{2,35,65,92} = 13.99$ ,  $P < 0.001$ ) with no *sequence* by *test-block* interaction ( $F_{2,72,76,19} = 0.26$ ,  $P = 0.83$ ). The rate of the fast, within-test improvements in speed (the slope of the linear trend) did not differ between the two sequences ( $F_{1,28} = 0.03$ ,  $P = 0.86$ ). However, the within-test variability in speed (i.e. SD) was significantly smaller for the T-FOS compared to the U-FOS ( $F_{1,28} = 7.21$ ,  $P = 0.01$ ). There was no significant effect of *group* or *group* by *sequence* interaction for slope or SD.



**Figure 6. Interhemispheric functional connectivity between ROIs within homologous cortical areas**  
 A, M1 – M1. B, SMA – SMA. C, PMd – PMd. Fisher-transformed correlation coefficients (CC) for each block (Block1 and Block2) and each sequence (T-FOS and U-FOS). Columns, CC averaged across participants; bars, SEM; \*Significant at  $P < 0.05$ , with false discovery rate correction.

**Table 3. Statistics of correlation analyses between the experience-related differences in the M1 activity and connectivity during the untrained-hand's performance**

The left M1, contralateral to the performing (untrained) hand					
	$\Delta$ CC M1 – M1	$\Delta$ CC left-M1 – left-SMA	$\Delta$ CC left-M1 – right-SMA	$\Delta$ CC left-M1 – left-PMd	$\Delta$ CC left-M1 – right-PMd
$\Delta$ CV-Act within the 'untrained' (left) M1	$r = 0.39$	$r = -0.08$	$r = 0.06$	$r = 0.01$	$r = -0.02$
	$P = 0.16$	$P = 0.77$	$P = 0.83$	$P = 0.97$	$P = 0.96$
The right M1, ipsilateral to the performing (untrained) hand					
	$\Delta$ CC M1 – M1	$\Delta$ CC right-M1 – right-SMA	$\Delta$ CC right-M1 – left-SMA	$\Delta$ CC right-M1 – right-PMd	$\Delta$ CC right-M1 – left-PMd
$\Delta$ CV-Act within the 'trained' (left) M1	$r = 0.70$	$r = 0.27$	$r = 0.44$	$r = 0.33$	$r = 0.67$
	$P = 0.01$	$P = 0.34$	$P = 0.11$	$P = 0.23$	$P < 0.01$
	$P_{cor} = 0.06$				$P_{cor} < 0.05$

$\Delta$ CV-Act, experience-related differences in the magnitude of activity;  $\Delta$ CC, experience-related differences in the strength of functional connectivity;  $P_{cor}$ , values corrected for multiple tests using Bonferroni adjustments.

## Discussion

The present study supports the notion that the 'trained' M1 is involved in the hand independent representation of a sequence of finger-to-thumb opposition movements acquired through unimanual training. Superior performance levels for the T-FOS, as compared to the U-FOS, were associated with a correspondingly greater activity within M1, bilaterally, when the two sequences were performed at the same fixed rate with the untrained hand. Moreover, this experience-related differential activity, within the 'trained' M1, was positively correlated with experience-related differences in the functional connectivity between (1) the two primary motor cortices, as well as (2) between the 'trained' M1 and the left (contralateral) PMd. However, there was no correlation between the experience-related differences in the connectivity measures between these two routes, suggesting two independent neural pathways for the transfer of information between the two primary motor cortices: interhemispheric right-M1 – left-M1 and right-M1 – left-PMd – left-M1 route.

Although the actual experience of the untrained hand with either of the two sequences did not differ, the performance of the T-FOS was significantly faster, more accurate and more stable compared to the performance of the U-FOS during the post-scanning performance test. However, during the scanning session, the differences in the rate of sequence executions were minimized by requiring participants to perform the component movements at a comfortable but externally paced rate. Thus, the differences in neural activity could not be directly related to faster task execution. Relying on a

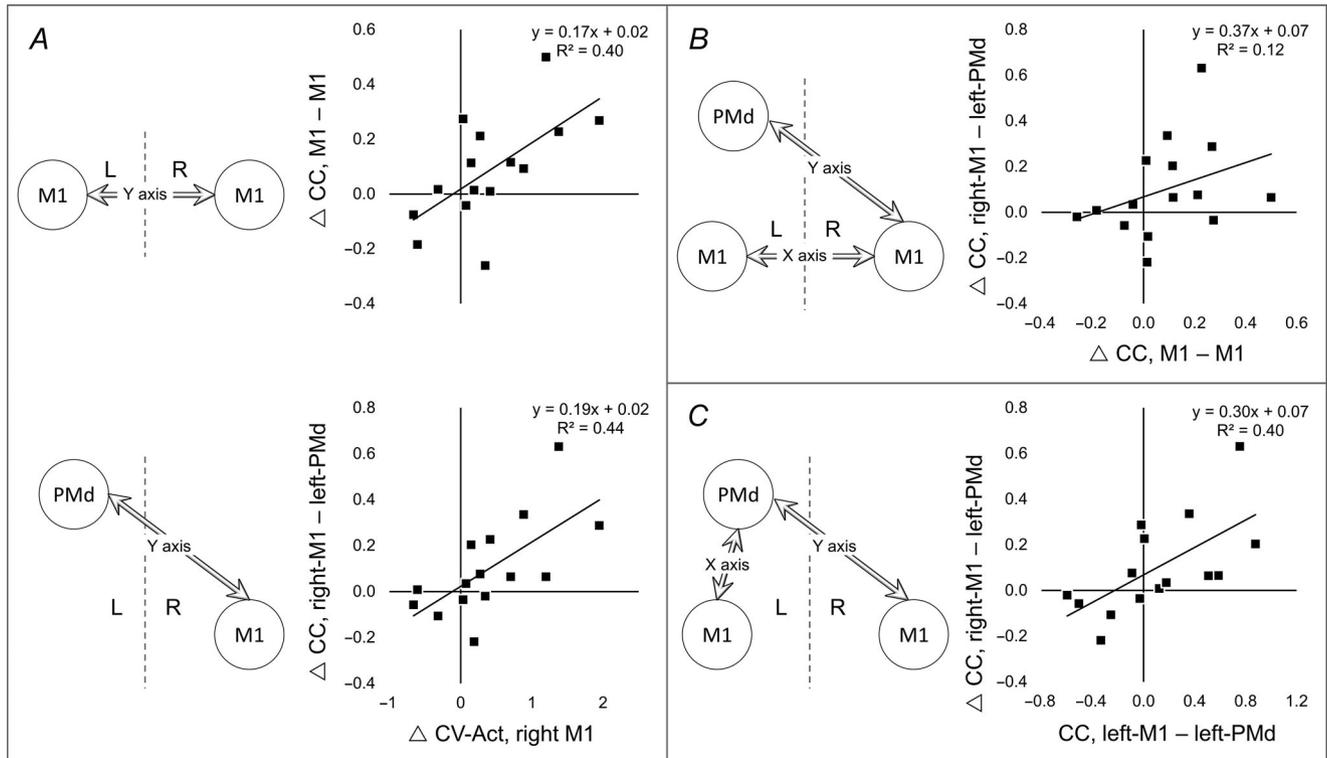
previously suggested notion (Karni *et al.* 1995), we argue that the neural activity and connectivity of M1 reflected differences in the order of the component movements within the movement sequence: trained *vs.* untrained. One day after the initial left-hand training, both primary motor cortices exhibited greater sequence-specific activity when the task was performed with the untrained hand (Figs 3 and 4). Thus, the 'trained' M1 showed a similar differential activity pattern to that observed in the 'untrained' M1 when the two sequences were executed by the untrained hand (i.e. predominately generated by the 'untrained' M1). The involvement of the 'trained' M1 in representing past experience with the T-FOS when it was executed with the untrained hand is further indicated by the finding that, across runs, neural signals within the 'trained' M1 consistently decreased during the U-FOS performance but not during the T-FOS performance (Fig. 4B). This pattern of results suggests that the contributions of the 'trained' M1 were diminishing as the U-FOS was repeatedly experienced, whereas its contribution to the performance of the T-FOS was similarly robust across runs.

Additional support for the idea that the 'trained' M1 is a locus of sequence-specific knowledge, and that this knowledge can be mobilized during the T-FOS performance with the untrained hand, is indicated by the differential across-block changes in the functional connectivity between the two primary motor cortices in the performance of the two movement sequences (Fig. 6A). Although there were no significant changes in functional connectivity upon the repeated blocks during the T-FOS execution, suggesting similar contribution of the 'trained' M1 during both performance periods, there

were significant across-block reductions in the functional connectivity between the two primary motor cortices when the U-FOS was repeated.

A number of TMS studies suggest that unimanual motor practice leads to task-related plastic changes not

only in the contralateral, but also in the ipsilateral M1 (Perez *et al.* 2007b; Duque *et al.* 2008; Lee *et al.* 2010). If the practice-driven changes in the organization of M1, ipsilateral to the trained hand, contribute to the improved performance of the T-FOS using the untrained

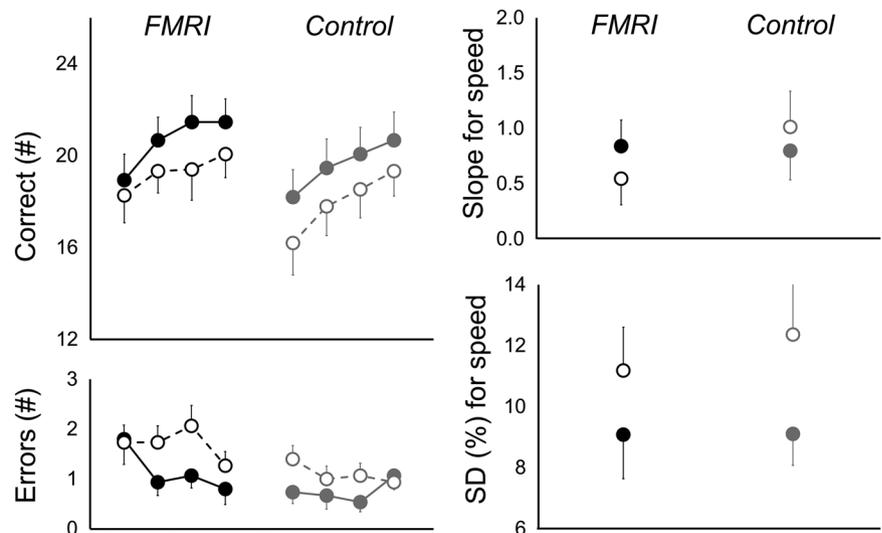


**Figure 7. Linear correlations of the experience-related differences in the M1 activity and connectivity**

A, experience-related differences in the strength of functional connectivity ( $\Delta CC$ ) between the two primary motor cortices (upper plot) and between the ‘trained’ (right) M1 and the left PMd (lower plot) are plotted vs. the experience-related differences in the magnitude of activity ( $\Delta CV\text{-Act}$ ) within the ‘trained’ (right) M1. Individual values of  $\Delta CC$  between the ‘trained’ (right) M1 and the left PMd are plotted vs. (B) individual values of  $\Delta CC$  between the two primary motor cortices and (C) individual values of  $\Delta CC$  between the ‘untrained’ (left) M1 and the left PMd.

**Figure 8. Behavioural results overnight**

Overnight performance measures of the T-FOS (filled circles and continuous line) and the U-FOS (white circles and dashed line) for the two experimental groups: fMRI (black markers) and control (grey markers) using the untrained (right) hand. The number of correctly completed sequences (upper left) and the number of sequences with ordering errors for each test-block (lower left), as well as within-test improvements in speed (slope, upper right) and variability for speed (SD, lower right), are shown. Data points, group mean values; bars, SEM.



hand, a relative reduction in neural activity to repeated motor experience, which presumably constitutes a robust indicator of novelty (Karni *et al.* 1995; Gabbitov *et al.* 2014, 2015), should be observed only for the U-FOS. However, in the present study, during the untrained-hand's performance, RS effects in the 'untrained' M1 were significant for both sequences within the initial blocks in each pair (i.e. not only for the U-FOS, but also when the T-FOS was continuously repeated) (Fig. 4A), suggesting that, for the 'untrained' M1, both sequences were novel. Similar RS effects in the 'trained' M1 were observed during the trained-hand's performance only for the U-FOS, but not for the T-FOS (Gabbitov *et al.* 2015). Thus, the results of the present study suggest that the implementation of sequence-specific knowledge during the intermanual transfer condition (i.e. performing the T-FOS with the untrained hand) does not reflect a newly acquired sensorimotor mapping within the 'untrained' M1 (which showed similar novelty-related RS effects for both sequences, T-FOS and U-FOS) but, instead, at least in part, is mediated through interhemispheric efferent connections from the 'trained' M1.

There is evidence that interhemispheric inhibition, induced by transcranial electrical cortex stimulation, is accompanied by the attenuation of the BOLD responses in M1 (Brocke *et al.* 2008). Therefore, in the present study, the increased activity within the M1 hand area, bilaterally, for the T-FOS, compared to the U-FOS, during the untrained-hand's performance, suggests that the interaction between the two primary motor cortices during the execution of the T-FOS was not an inhibitory one. Thus, the notion of an inhibitory role for the 'trained' M1 in the retrieval of the previously acquired motor-skill using the untrained hand (Romei *et al.* 2009) is not supported. Instead, the results of the present study suggest that the transfer of sequence-specific information between the two primary motor cortices is predominantly mediated by excitatory mechanisms driven by the 'trained' M1. Moreover, the experience-related increases in the intensity of the BOLD signals exhibited by the 'trained' M1 were positively correlated with experience-related differences in M1 – M1 functional connectivity (Fig. 7); however, no correlation was found between activity within the 'untrained' M1, contralateral to the performing hand, and M1 – M1 functional connectivity. These results support the notion that the interhemispheric M1 – M1 functional connectivity reflects the 'trained' M1 contributions for differentiating between the two sequences.

We propose that the current pattern of results cannot be explained (exclusively) by assuming a reduction in interhemispheric M1 – M1 inhibition. In humans, direct, short latency (Ferber *et al.* 1992) M1 – M1 transcallosal connections are mainly considered to be inhibitory (Ferber *et al.* 1992; Meyer *et al.* 1995; Hanajima *et al.* 2001) and, presumably, suppress simultaneous bilateral

activation of M1 to prevent redundant, mirror movements in the resting hand rather than control motor actions in the active hand (Kobayashi *et al.* 2003). Long-latency interhemispheric inhibition may be mediated by different neural circuits (Chen *et al.* 2003; Chen, 2004; Ni *et al.* 2009; Uehara *et al.* 2014), perhaps involving premotor areas (Boudrias *et al.* 2012). However, there is no good evidence to support the notion that such connections are related to intermanual transfer effects. Perez *et al.* (2007), when studying the effects of motor sequence training on functional changes in M1 using TMS, have shown that the immediate post-training reductions in the amount of the short-latency (10 ms) interhemispheric inhibition driven by the 'trained' M1 were correlated with non-specific performance improvements in the untrained hand but not with sequence-specific intermanual transfer. Talelli *et al.* (2008), when exploring the interhemispheric interaction of the two primary motor cortices, failed to show correlations between the short-latency (10 ms) interhemispheric inhibition and the task evoked BOLD signal in motor areas of interest, including the M1. However, the increased activity within the M1 ipsilateral to the performing hand was associated with reduced M1 – M1 long-latency (40 ms) interhemispheric inhibition (Talelli *et al.* 2008; Boudrias *et al.* 2012). Thus, rather than inhibition modulation, it was suggested that the right M1, ipsilateral to the active hand, plays a central role in maintaining performance levels through increasingly facilitatory corticocortical influences (Talelli *et al.* 2008; Boudrias *et al.* 2012).

In the present study, when the two movement sequences were performed with the untrained (right) hand, the experience-related increases in the intensity of the BOLD signals, exhibited by the 'trained' M1, were also positively correlated with experience-related differences in the functional connectivity between this region and the left (contralateral) PMd (Fig. 7). No such relationship was evident for the experience-related differences in M1 – PMd functional connectivity within the 'trained' hemisphere. Moreover, the differences in the interhemispheric connectivity between the 'trained' M1 and the contralateral PMd were positively correlated with the differences in M1 – PMd intrahemispheric connectivity within the 'untrained' hemisphere. Highly consistent activation of the left PMd in various unimanual motor tasks performed with either hand was reported by a recent meta-analysis of 70 fMRI experiments (Hardwick *et al.* 2013). The left PMd may be a critical node in the motor learning network irrespective of performing hand, guiding both motor cortices, in line with a putative dominant role of the left hemisphere in the control of movement intended for both sides of the body (Serrien *et al.* 2006). Our results suggest that the left PMd is not only recruited in the planning and control of specific actions, but also may be involved in the retrieval of sequence-specific knowledge

acquired during training with the ipsilateral hand. This knowledge is presumably utilized during the generation of the T-FOS with the untrained hand. The present study cannot provide direct evidence for or against a dominant role of the left PMd in learning and retention of a sequence of movements irrespective of the hand used for practice because transfer was tested only in one direction (from a trained left to an untrained right hand). The results of the present study suggest that the interhemispheric interaction between the 'trained' M1 and the contralateral PMd may be mediated via a third brain region, other than the homologue of the PMd within the 'trained' hemisphere or through the few heterotopic callosal connections between M1 and PMd (Marconi *et al.* 2003).

There was no correlation between the interhemispheric M1–M1 and 'trained' M1–contralateral PMd differential connectivity measures. This pattern of results suggests that the 'education' of the 'untrained' M1 may be mediated through two independent neural pathways subserving the transfer of information between the two primary motor cortices. The existence of an interhemispheric M1–PMd anatomical pathway, which is distinct from the interhemispheric M1–M1 connections, was suggested by conditioning TMS studies (Mochizuki *et al.* 2004; Bäumer *et al.* 2006), as well as by an fMRI study of stroke patients (Bestmann *et al.* 2010).

The results of the present study provide no evidence in support of the involvement of the SMA in the intermanual transfer of the previously practised sequence of movements. The SMA was suggested to be a locus representing motor sequences, as acquired during the training on the serial reaction time (SRT) task (Nissen & Bullemer, 1987), that can be accessed by both hands when performance is tested immediately after training (Grafton *et al.* 2002; Bischoff-Grethe *et al.* 2004; Perez *et al.* 2007b, 2007a, 2008). In the FOS task, the SMA showed RS effects only for the U-FOS when performed with the trained hand (Gabitov *et al.* 2014). In the present study, during task performance using the untrained hand, the RS effects in the SMA across blocks were also significant only for the U-FOS (Fig. 5A). However, there was no correlation between the connectivity of the SMA with the M1, bilaterally, and the differential activity in either the 'trained' or the 'untrained' M1. It may be that the SMA has a critical role in sequence representation only during and immediately after training on a novel movement sequence. This may not hold true for a consolidated movement sequence. There are data suggesting that, in monkeys, the contributions of the SMA to the performance of movement sequences can be markedly overtaken by the M1 after extensive practice (Lu & Ashe, 2005). The complexity of a motor task and previous experience may determine the time-scale of plasticity and reorganization of brain representations (Ashe *et al.* 2006). The individual opposition movements are well

established in young adults and may have contributed to the relatively rapid shift of motor sequence representation from associative to sensorimotor circuits (Doyon & Benali, 2005). The results of the present study therefore indicate that a single consolidation interval may suffice in effecting a shift from a higher level SMA-related to a lower level M1-dependent representation of the movement sequence.

We speculate that, in addition to possible direct M1–M1 and M1–PMd commissural connections (Mochizuki *et al.* 2004; Bäumer *et al.* 2006; Ni *et al.* 2009), the transfer of sequence specific knowledge between the two M1s might be mediated through a corticostriatal loop. Studies on human subjects and non-human primates suggest that the corticostriatal loop is crucial for mediating the acquisition and execution of movement sequences (Alexander *et al.* 1986; Tanji, 2001; Hikosaka *et al.* 2002; Doyon & Benali, 2005), as well as the modification of previously established movement sequence knowledge (Censor *et al.* 2014). The motor circuit of the striatum receives substantial, somatotopically organized projections from the primary motor cortex, as well as from the frontoparietal regions implicated in motor actions (Middleton & Strick, 2000), and therefore is considered to be involved in the integration of multiple sources of information for generating an appropriate action. Animal studies indicate bilateral corticostriatal connections originating from each M1 (Faraji & Metz, 2007; Alloway *et al.* 2009).

## Conclusions

The results of the present study suggest that the representation of a movement sequence (extensively trained 1 day before) within the 'trained' M1 contributes to the ability of the 'untrained' M1 to generate the corresponding movement sequence with the untrained hand. This interhemispheric transfer of sequence-specific knowledge is predominantly mediated by excitatory mechanisms driven by the 'trained' M1 via at least two independent neural pathways.

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## Additional information

### Competing interests

The authors declare that they have no competing interests.

### Author contributions

EG, DM and AK were responsible for the conception and design of the study. EG and AK were responsible for analysis and interpretation of data. EG and DM were responsible for drafting the article. AK was responsible for critical revision with respect to important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

### Funding

EG was partially supported by a fellowship from the EJ Safra Brain Research Centre for the Study of Learning Disabilities. The research leading to these results has received funding from the European Union Seventh Framework Program (FP7/2007–2013) under grant agreement no. 604102 (Human Brain Project).

### Acknowledgements

We thank Tammi Kushnir PhD, Director of Research at the C. Sheba Medical Centre, for her technical and administrative support.