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# Increased functional connectivity is crucial for learning novel muscle synergies

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To gain efficiency in performance of a novel complex movement, we must learn to coordinate the action of the pertinent muscle groups. We used functional magnetic resonance imaging (fMRI) to investigate the mechanisms of learning a novel synergic movement in human primary motor cortex (M1). We show for the first time changes in connectivity profiles between muscle representations in relation to learning and short-term plasticity. The abductor pollicis brevis (APB) and the deltoid muscles were trained for fast synchronous co-contraction. This learned synchrony of muscle contractions was related to rapid increase in functional connectivity between the central M1 representations of the participating muscle groups. Directionality and size of use dependent plasticity shifts in APB muscle representation in M1 also showed links to performance of the task and general levels of daily activity. This result suggests that functional connectivity between MI representations of participating muscle groups are a basic central mechanism for establishing movement synergies. The timing of the increased connectivity and directional nature of the plasticity provide insight into the cortical integration of M1 muscle representations as a function of lifestyle and learning processes. Greater levels of daily activity may increase the integration of muscle representations across the motor cortex, enabling faster learning of novel movements. © 2007 Elsevier Inc. All rights reserved.

#### Introduction

How can we, despite the great number of participating muscles, make smooth, economic and well coordinated movements? Integration of the muscle movements takes place in both M1 cortical circuits within the human brain (Devanne et al., 2002) as well as within the spine. The somatotopic map of M1 has been investigated thoroughly and is anatomically well described. Using tracer injections of physiologically identified sites, far reaching intracortical collaterals are observed terminating in 'clusters' up to 8 mm from injection sites (Huntley and Jones, 1991). Clusters controlling various forelimb segments and antagonistic muscles are heavily interconnected by intrinsic horizontal collaterals (Capaday et al., 1998). The clusters, or in this case termed 'colonies', of cortico-spinal motor neurons are also described after microstimulation mapping of multiple forelimb muscles of the cat (Schneider et al., 2002). Similar results were first seen by microstimulation mapping of the squirrel monkey (Donoghue et al., 1992) and were described as 'intermingling' of muscle representations. A patchy, mosaic pattern of muscle representation appears to be the main consensus finding from such studies and this patterning presumably facilitates cortical integration of muscles for movement. But still, how distantly located muscle representations interact to bring about novel synergic movement is not yet known.

Muscle synergies are considered as coherent activations in space or time by a group of muscles and are suggested as the building blocks for complex movements (d'Avella et al., 2003). Based on animal experiments, muscle synergies have been shown to be encoded within primary motor cortex (Holderfer and Miller, 2002). We aimed to show for the first time changes in functional connectivity between cortical muscle representations in humans. The short time span for remapping that occurs after peripheral nerve lesion strongly suggests that mechanisms for changing functional connectivity across the cortex exist (Donoghue and Sanes, 1988; Donoghue et al., 1990). Changes in functional connectivity within the animal M1 may therefore underlie the learning of novel synergic movements. The hypothesis that connectivity is established by disinhibiting previously existing cortical circuits (Jacobs and Donoghue, 1991) was later demonstrated in the cat motor cortex (Schneider et al., 2002).

*Abbreviations:* APB, Abductor pollicis brevis; M1, Human primary motor cortex; MOA, Muscle onset asynchrony.

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fMRI data offer the opportunity to measure functional connectivity *in vivo* in humans (Kelly and Garavan, 2005) and has previously been used by our group to demonstrate tight correlations of performance and learning to increases in connectivity between brain areas in humans within differing networks (Buechel et al., 1999; Wolbers et al., 2006). The definition of functional connectivity used here is the 'correlation between spatially remote neurophysiological events' (Gerstein and Perkel, 1969), and neither implies nor rules out 'direct' (effective) connectivity between regions. In brief, this study identifies subjects muscle representations for two muscles and computes the correlation coefficients in activity between representations. Comparisons are made between the correlation coefficients obtained before and after learning a motor task, consisting of co-contraction of distal (APB) and proximal muscles (deltoid muscle).

One way of studying learning of muscle synergies is the investigation of co-contraction between muscle groups during training of novel movements. As the co-contraction task is performed it becomes a smoother and more singular synergic movement. Such tasks have been widely used in research with humans and with the advent of transcranial magnetic stimulation (TMS). These studies have already revealed a 'use dependent plasticity' in M1 (Classen et al., 1998; Liepert et al., 1999; Tegenthoff et al., 1999). Together they demonstrate that after learning of a novel co-contraction movement, the centre of gravity for the representation of one muscle shifts towards that of the second, co-contracted muscle group. Evidence that Hebbian type binding could underlie the phenomena is wide ranging (Ziemann et al., 2001, 2004; Stefan et al., 2005), including the manipulation of *in vivo* γ-aminobutyrate (GABA) affecting this 'use dependent plasticity' (Stefan et al., 2002). Also using TMS in humans, associative stimulation, simulating paired muscle synergies is blocked by an N-methyl-D-aspartate (NMDA) antagonist, indicating that a long-term potentiation (LTP) like process plays a role in human M1 motor learning (Ziemann, 2004).

We therefore hypothesized that functional connectivity between the muscle representations and the shifts of muscle representations toward each other are associated with the improved performance in synergistic co-contraction of participating muscle groups. Our goal was to measure this connectivity for the first time in the human and to determine what relationship, if any, it has with the shifts in representations observed during previous TMS studies. We expected that the improvement of muscle onset synchrony (MOA) may be bound to increased functional connectivity between the M1 representations of the participating muscle groups and possibly to concomitant shifts of representations.

#### Materials and methods

## General procedure

We implemented a simple fMRI paradigm in which BOLD signal was used to localize muscle representations prior to and post cocontraction training (Fig. 1). fMRI acquisitions consisted of a fast event-related design, where events belonged to one of four conditions, 'contraction of APB only', 'contraction of deltoid only', 'contraction of deltoid and APB as synchronously as possible', 'no movement'. An event is therefore a single instance of one of these movements. The movement required of the subject was indicated by a number (1-4) centrally displayed on a screen to the subject for 1 s via a mirror. The movements are shown in the photograph in Fig. 1. On the left a subject is shown resting, arrows indicate the required movement of (1) the thumb using the APB and (2) the arm using the deltoid muscle. The picture on the right in Fig. 1 shows the final position of the subject under condition 3 (synchronous abductions). Each condition consisted of 20 events. The co-contraction event, condition 3, was then trained outside of the scanner for a 40-min period (120 events) with the task of reducing muscle onset asynchrony. A second identical fMRI session was conducted immediately after training. Peak point BOLD signal corresponding to APB of deltoid movements were localized for each subject individually. The time courses extracted at each individual's APB representation and deltoid representation and a correlation coefficient between these two time courses found and used as a measure of functional connectivity. Comparisons of connectivity were made between sessions (before vs. after learning) as well as analysis of the shifts in the peak point of muscle representation. Finally, post hoc detailed questionnaires about subject's lifestyle in terms of daily activity were also implemented in the analysis.

#### Participants

Twenty-three right-handed volunteers underwent successful data acquisition under the protocol approved by the local ethics committee. Two subjects were removed from the analysis due to



Fig. 1. Paradigm design: fMRI experiments consisted of four conditions: (1) APB abduction, (2) deltoid abduction, (3) synchronous abduction of APB and deltoid or (4) no movement. Numbers presented on a screen and viewed via a mirror indicated to the subject the movement to be undertaken. fMRI measurements were conducted prior to and post a 40-min training of condition 3 with the task to minimize muscle onset asynchrony. On the left the direction of required movement, (1) APB, (2) deltoid. On the right the final body position after correct performance of condition 3. Photos are adapted from Pleger et al. (2003) (41) with kind permission of Springer Science and Business Media.

head movements of greater than 1 mm. Moreover, two subjects were removed from the analysis due to having inverted performance curves, leaving 19 ( $26.6\pm3.6$  years; 16 male, 3 female) included in the analysis. Participants playing instruments or with extensive sports participation were not included in the study.

#### Procedure

We employed a simple co-contraction training paradigm as published previously (Pleger et al., 2003) which was flanked by fMRI data acquisitions (Fig. 1). Training was conducted outside the scanner.

#### Co-contraction movement

A fast abduction of the thumb simultaneously with abduction of the deltoid (Fig. 1). An isolated abduction of the thumb is performed extremely seldom and is mediated mainly by the abductor pollicis brevis muscle. The deltoid movement was a quick short range abduction of the upper arm (mainly mediated by the mesial part of the deltoid muscle). Both movements involved joint motions, but the range of upper arm movement was minimal. Movements were preferentially mediated by APB and deltoid; however, concomitant activation of neighboring muscles cannot be avoided. The movement was well circumscribed; therefore, it is very unlikely that there was minimal recruitment or de-recruitment of other muscle groups. Isolated abduction of the thumb, simultaneously, and solely, with the abduction of the deltoid does not belong to the human everyday motor repertoire. This new combined simultaneous movement must be learned.

#### Co-contraction training

Participants were trained in the co-contraction motor task described above consisting of co-contraction of the deltoid and APB muscles. Over a 40-min training period participants attempted to contract the two muscles as synchronously as possible after the "ready, steady, GO" instruction of the experimenter. To evaluate the time course of motor learning, co-contractions (three repetitive movements per minute) had to be performed during 4 sessions (s1-s4) over a period of 40-min (0-10 min, 10-20 min, 20-30 min, 30-40 min), resulting in a total number of 120 movements. As a behavioral measure of co-contraction absolute time differences between the onsets of the contraction of both muscles, termed "muscle onset asynchrony" (MOA) were recorded using surface electrodes connected to conventional EMG (electromyography) equipment (Dantec Cantata, Denmark). The subjects were given verbal feedback about their MOA after each movement. EMGs were recorded continuously and stored for off-line analysis using Spike2 (Version 5.00).

# fMRI task and stimulus presentation

Fast event-related fMRI was performed in all subjects before and after the co-contraction training (Fig. 1). During fMRI, participants were visually presented with a pseudo-random series of numbers (one to four) of which they had been instructed to perform one of four tasks with their right hand/shoulder: (1) contract the deltoid muscle alone, (2) contract the APB alone, (3) contract the deltoid and APB as synchronously as possible or (4) do not move. Typically in pilot studies movements are completed well within 1 s after cue onset. Post-training participants underwent a second fMRI scan using the identical paradigm. Presentational timing of stimuli was controlled from a separate PC using "Presentation" (Neurobehavioural Systems) software. Numbers to cue movements were presented (light blue on dark blue background) for 1 s, with a jittered inter-stimulus interval of mean 4 s,  $\pm 2$  s during which a fixation cross was shown. Twenty events from each of the four conditions were presented over approximately 5 min, 30 s. During fMRI, subjects performed twenty movements for each of the four conditions. In contrast to the training phase, during fMRI acquisition, EMG recordings and consequently feedback to the subject regarding performance was not available.

#### fMRI data acquisition

Scanning was conducted on a 3-T system (Siemens Trio) with gradient echo EPI T2\*-sensitive sequence, using a standard head coil. Contiguous gradient echo, echo-planar images in thirty-four 3-mm thin slices without gap, interleaved acquisition, TR 2 s, TE 25 ms, flip angle  $80^{\circ}$ . Slices covered the entire brain positioned parallel to the plane intersecting the anterior and posterior commissure. The matrix acquired was 64\*64 with a FOV 192\*192 mm, voxel size 3\*3\*3.

#### fMRI data analysis

fMRI data were analysed using SPM2 (Welcome Department of Cognitive Neurology, London, UK) and MATLAB 6 (The Mathworks Inc.). Pre-processing was employed, in which the first four images were discarded to remove out of phase measurements. The subsequent data series was realigned to the first volume (no slice time corrections), normalized to MNI (Montreal Neurological Institute) standard space and spatially smoothed using a Gaussian filter of 6 mm FWHM, i.e., at twice the voxel size (Worsely and Friston, 1995) prior to conducting event-related analysis. Four conditions were modelled using a canonical hemodynamic response function. Data were high-pass filtered to remove low frequency artefacts. First level analysis of each individual was conducted with four regressors, one for each of the four conditions. One-tailed Student t-tests were used to identify brain regions most responsive for APB flex only and deltoid flex only (conditions 1 and 2).

#### Localizing peak points

Masks for identifying hand and deltoid motor representations were drawn point by point (see Supplementary Figure) on the MNI (Montreal Neurological Institute) single subject template brain using the MarsBaR tool (http://marsbar.sourceforge.net). The APB mask encompassed the hand knob region (z48-z69) of the left hemisphere primary motor cortex. The deltoid mask lay along the pre-central gyrus between z coordinates 63 and 77. Using these masks, peak point BOLD signal was identified from the main effect of 'APB only' or 'deltoid only' flexing conditions (threshold t < 0.001 uncorrected). We localized APB and deltoid neural representations using peak BOLD activity independently for preand post-training sessions for each participant individually. Peak point activation was identified using the main effect of contracting the APB only or of the deltoid only. Peak points for both APB and deltoid were selected after application of the masks described above. Multivariate analysis of covariance was conducted to identify significant shifts in BOLD peak point activity for APB and deltoid representations between sessions. ROIs were 6 mm in diameter and therefore included 7 voxels, which never impinged upon one other. We deemed small ROIs as both necessary due to the spatial proximity of the muscle representations but not detrimental to analysis based on reports (Gonçalves and Hall, 2003) in assessment of structural equation modelling. Results of which indicate little difference between using large and small ROI's. They conclude large ROIs while giving generalized results may fail to capture functional differences within specialized areas.

#### Functional connectivity analysis

Preprocessed smoothed BOLD signal for the entire acquisition was extracted for each ROI for each participant. This BOLD time course was deconvolved to provide an estimate of the neural time signal behind the BOLD response (Friston et al., 1997; Gitelman et al., 2003). As this is only an estimate at best of the neuronal signal, we only report data which also passed significance at p < 0.05independent of the deconvolution step, i.e., when identical analysis was carried out directly upon the fMRI BOLD time series. Correlation between the two estimated neuronal time courses from the APB and deltoid ROIs were used as a measure of functional connectivity. Pearson's correlation coefficients of the pre-training neural signals between 'APB pre-training'-'deltoid pre-training' were calculated as were the post-training neural signals using posttraining ROIs. Thereafter, each subject had a single correlation coefficient score between the two representations both before and after motor learning. Pearson's correlation coefficients not being normally distributed were subjected to a Fisher transform prior to statistical analysis. Dependent t-tests were used to compare between sessions for each group and across all subjects. The Fisher transform was reversed prior to plotting to give readers an idea of the correlation coefficients that we found between our ROIs. To demonstrate the relationship of distance between APB and deltoid representations to connectivity (Fig. 3b), Spearman's non-parametric correlation coefficients are presented.

#### Results

# Effects of training

Within the group the mean decrease in MOA during learning to co-contract APB and the deltoid was 16.58 ms ( $\pm$ 9.11 ms SEM; repeated measures ANOVA with factor SESSION (s1–s4): *F*(1,18)=109.120; *p*<0.001). The distribution of the data indicated two types of participants which we termed 'early

efficiency (EE)' and 'late efficiency (LE)' as they differ in the length of time required to acquire minimum MOA and hence 'efficient' performance (Fig. 2a). This was due to differences in both initial performance and a floor effect of performance. Therefore, the groups were split into two on the basis of their decrease in MOA being above or below the arithmetic mean. Late efficiency participants acquired efficiency over the period of the training sessions. We found a significant difference in the mean decrease of MOA between both groups (univariate ANOVA with factor GROUP: F(1,17)=39.107; p < 0.001).

The late efficiency group (n=8) showed a significant gain in performance as indicated by a decreased MOA during learning the task (repeated measures ANOVA with factor SESSION (s1-s4) F(3,21)=28.460; p<0.0001). Mean reduction in MOA was 25.25 ms±6.11 ms between their first 10 min (s1) of training and last 10-min of training (s4). The early efficiency group (n=11)showed a far smaller but significant gain of 10.27 ms±4.41 ms over the same period (repeated measures ANOVA with factor SESSION (s1-s4) *F*(3,30)=21.109; *p*<0.0001). Within group, post hoc Scheffé tests comparing each 10-min session to the following 10-min session demonstrated learning between each session (s1-s4) for each group (early vs. late efficiency group) at p < 0.01. A direct comparisons between the groups revealed that the late efficiency group was considerably slower than the early efficiency group within the first 10 min (session 1; paired t-test p < 0.005). However, this difference in learning was reduced to a significance level of p=0.06 (paired *t*-test) between second 10-min sessions.

We localized APB and deltoid neural representations independently for pre- and post-training sessions for each participant by identifying peak point activation to, respectively, the main effect of contracting the APB or deltoid only. Peak points for both APB and deltoid were selected after application of a mask of primary motor cortex. The time course for each of these voxels was averaged with all voxels from the ROI and used for further analysis. A measure of functional connectivity between the APB and deltoid regions of interest (ROIs) for each participant before and after motor learning was taken by comparing correlation coefficients between these two peak BOLD signals of both points (see methods for details).



Fig. 2. (a) Behavioural data: bar chart of group mean MOA in the first and last 10 min of training. The late efficiency group achieved the early efficiency groups initial level of MOA after 40-min training. Error bars=standard error mean. See text for full statistical description of data. Note that the *y* axis is inverted so that *increasing performance* is illustrated. (b) Illustration of connectivity between APB muscle representations. Thickness of line denotes levels of functional connectivity between regions for each group (red dashed=pre-training late efficiency, green dashed=late efficiency post-training, red solid=pre-training early efficiency). Bar chart shows the illustrated connectivity data (Pearson correlation coefficients) between these APB/ deltoid regions in each group, error bars=standard error mean. Asterisk denotes significant difference p < 0.01 between sessions (two-tailed dependent *t*-tests on fisher transformed correlation coefficients).

#### Functional connectivity

We tested for learning dependent changes in functional connectivity between the APB and deltoid muscle representations within each of our two groups. The late efficiency group showed an increase in functional connectivity between APB and deltoid M1 motor representations post-training compared with pre-training (two-tailed dependent *t*-tests, p < 0.013, n=8), see Fig. 2b. This means that as a novel synergy is learnt then the functional connectivity of the cortical regions involved in the independent muscle movements increases. This occurred only for our late efficiency group yet it can be seen that the early efficiency group had a high, unchanging level of connectivity both pre- and post-training perhaps giving rise to their early efficiency in producing the synergy.

Given the fact that the APB and deltoid representation within MI was spatially separated by just a couple of centimeters (mean 17 mm, SEM 1.4), a strong relationship of increasing connectivity with reducing distance would be expected. This expectation is due to factors such as the spatial smoothing of data (see Materials and methods) and the inherent intracortical connections which may stretch up to 8 mm from a single pyramidal neuron (Huntley and Jones, 1991). As early efficiency learners peak points moved closer to one another, we performed additional analysis of the data in order to rule out that the increase in connectivity is not merely a consequence of this shift. This was tested in two ways. First we tested the assumption that connectivity increased with reducing distance between regions. We calculated connectivity of every voxel in M1 to the APB peak point for each subject and plotted the results as a function of distance from the seed voxel (Fig. 3a). We found increasing connectivity as the distance between voxels reduced. We noted that early efficiency learners and late efficiency learners post-training did not correspond with predictions based purely on distance, i.e., they do not fall upon the curve predicted by distance alone but had connectivity greater than expected. Secondly, to test formally whether the reported changes in functional connectivity were not merely a trivial consequence of reducing distance between representations, Spearman's rank correlation coefficients of distance between peak points and functional connectivity were calculated (Fig. 3b). As expected, prior to training there was a highly significant relationship of distance between APB and deltoid representations to functional connectivity levels (Spearman's R=-0.56, p=0.012, n=19). The effect, however, was mainly carried by the late efficiency group (Spearman's R=-0.79, p=0.021, n=8) and can be clearly observed as the open circle in Fig. 3a. The relationship of distance and connectivity seen here was severely eroded by the process of training. Across the groups the drop was substantial (Spearman's R = -0.33 (from -0.56), p = 0.16 (from -0.012), n = 19). Yet again, however, the late efficiency group was mainly responsible for this effect with dramatic ablation of the original relationship (Spearman's R=-0.34 (from -0.79), p=0.41 (from -0.021), n=8). Training had little effect on the early efficiency participants which pre-training had, at best, only a slight trend towards a relationship and post-training just slightly less so, see Fig. 3b.

If increasing connectivity was a direct consequence of increased synchrony of motor neuron firing then levels of connectivity should be directly correlated to MOA. Fisher transformed values were tested for direct correlation (Pearson's *R*) with MOA, with all subjects both sessions together, n=38, 2 degrees of freedom, p>0.5 for all three connectivities. As such this cannot be described as the principle cause for the observed rise in connectivity. Tests were



Fig. 3. Demonstrating increased connectivity is not an artifact of reducing distance. (a) Normal 'general' negative correlation of connectivity and distance between a seed and surrounding voxels. Connectivity with seed voxel and distance from the seed voxel was calculated for each other voxel in the motor cortex in each subject (dots). Error bars indicate standard error mean of all measurements (>17) taken at that distance from seed voxel. The mean distances between the seed voxels used to calculated the reported results and the mean connectivities are also plotted. Note late efficiency learners lay close to the curve pre-training (circle) but not after training (cross). (b) Reduced relationship of connectivity 'specifically' between APB and deltoid representations with the distance (mm) between APB and deltoid muscle representations post-training. The expected strong relationship seen pre-training (circle in panel a) is eroded by training (cross in panel a), particularly in the late efficiency group. Spearman's rank correlation coefficients are given on the 'y' axis. Group sizes: all (n=19), late efficiency (LE) (n=8), early efficiency (EE) (n=11).

made for differences between EMG amplitudes, durations and area under curve with between first and last 10 min of training within subjects (repeated measure ANOVAs with factor SESSION) and between subjects (mixed repeated measure ANOVAs with factor SESSION and the non-repeated measure factor GROUP). No SESSION effects were found. However, early efficiency EMG recordings from the APB muscle indicated greater amplitudes than late efficiency data (mixed repeated measures ANOVA with factor GROUP F(1,16)=5.564; p<0.034). This was partnered with an increase in the area under the curve (mixed repeated measures ANOVA with factor GROUP F(1,16)=6.282; p<0.023). Finally, and importantly, no significant correlations were found between functional connectivity measures and EMG amplitude, duration or area under the curve. The relationship was limited to muscle onset asynchrony.

#### Plasticity

Previous work (Classen et al., 1998; Liepert et al., 1999; Tegenthoff et al., 1999) suggests that the shift in locus is directional with the dominant use muscle (APB) shifting towards the second muscle representation. Shifts of muscle representations towards that of the APB have not been reported. In our case, we therefore expected a dorsomedial shift towards the deltoid representation. In our experiment the groups diverged in terms of their shift in locus of APB peak BOLD signal as well as in their functional connectivity (Fig. 4). The late efficiency group showed a significant (MANCOVA, F(3,5)=44.3, p<0.01), mainly medial but slightly caudally directed shift in peak point activation (mean Euclidean distance=7.68 mm). This result was more or less as expected, a shift towards the deltoid representation. This result was paired with a reduced Euclidean distance between the deltoid and APB representations post-training compared to pre-training (p < 0.05). Early efficiency groups on the other hand demonstrated similarly sized shifts of the APB representation (mean Euclidean distance=6.23 mm) but in no specific direction (MANCOVA, F (3,8)=0.7, p=0.54). No significant shifts were identified in the analysis of deltoid representations.

#### Lifestyle

Group results diverged in terms of directionality of shift as well as the relationship of connectivity to performance. Thus, we tested the further hypothesis that levels of daily activity were impacting on the arrangement of motor representations within the M1 cortex. Post hoc, participants were therefore asked to complete the International Physical Activity Questionnaire (www.ipaq.ki.se). The graph in Fig. 4 shows the negative relationship between distance of shift of APB representation between sessions and the subjects daily levels of activity measured as Metabolic Equivalents (MET) score, p < 0.05.

#### Discussion

The novel finding of this study is that significant reduction of the muscle onset asynchrony in the course of learning to cocontract previously untrained muscle groups was associated with a significant increase in functional connectivity between the representations of these muscles in M1. This result implies that establishing functional connectivity between the M1 representations of the participating muscles is a crucial process underlying learning new muscle synergies.

In more detail, a significantly larger rise in functional connectivity between the relevant muscle representations in M1 has been found only in the late efficiency group and its participants showed greater improvement in performance as rated by MOA. No



Fig. 4. Red dots=pre-training peak points, green circles=post-training peak points, red cross=mean of pre-training points, green cross=mean of post-training points, white cross hair indicates standard deviation from the mean. (a) Late efficiency APB peak points, z=57. Note general medial shift. (b) Early efficiency APB peak points, z=58. (c) Late efficiency deltoid peak points, z=68. (d) Early efficiency deltoid peak points, z=68. (e). Results of a post hoc (n=15) questionnaire of daily activity measured in metabolic equivalent ratings (METS). It shows a negative correlation (p<0.05) between daily activity and distance of shift of APB representation.

other EMG criteria showed similar group differences or correlations to connectivity. However, the early efficiency participants did not show changes in connectivity but instead had high connectivity even in the first few trials in which they were scanned. There are parallels to be drawn here with a previous study showing greater connectivity within the motor system when learning novel movements than after learning of these movements (Sun et al., in press). In that study, connectivity was measured between sensorimotor cortex, premotor cortex and the supplementary motor area. However, here we show this relationship occurs across short distances between specific motor representations. The distance between representations fell in the late efficiency group but it is not this reduction in distance that increases the connectivity. On the contrary, even though representations moved closer to one another post-training, the relationship of increasing connectivity with decreasing distance is removed. Furthermore, in early efficiency subjects where connectivity is high from the outset, we find the relationship of distance and connectivity is never significant. Although increasing correlations with distance does play a role, it is clear that functional connectivity between representations is strongly affected by training in late efficiency subjects. Based on the presented data, shifts can be accurately described as, 'to the most efficient locus for mediating the new synergy'.

Use of other interacting neural regions such as the SMA for coordinating activity may also take a role, but there is no a priori reason to assume that such a mediator is necessary. We suggest that it is plausible that M1 cortico-cortical interactions may be capable of directly mediating the ramping up of functional connectivity seen here via GABA mediated inhibition (Capaday, 2004). Further studies, in which pharmacological intervention in the learning process is implemented, will elucidate further the means by which the synaptic mechanisms of functional connectivity are increased. Our early efficiency participants might have rapidly disinhibited previously existing circuits while late efficiency participants require a more time consuming establishment of LTP.

The finding of increased connectivity and directional shifts differing between groups based on behavioral data requires us to speculate as to why such directional shifts may occur. Intracortical electrical stimulation mapping clearly shows muscle representations to have considerable overlap in M1 cortex (Kwan et al., 1978; Sessle and Wiesendanger, 1982; Gould et al., 1986; Donoghue et al., 1992; Nudo et al., 1990; Devanne et al., 2006) with somatotopy being maintained at the gross level but breaking down at the finer scale (Sanes and Donoghue, 2000). Imaging studies concur, although peak points of 'dominance' are often still observed as somatotopically arranged, but highly overlapping (Lotze et al., 2000; Kleinschmidt et al., 1997; Park et al., 2001). Additionally, increased use of muscles is known to expand their area of cortical representation in humans (Pascual-Leone et al., 1993; Tyè et al., 2005). This presumably increases levels of integration and overlap as demonstrated electro-physiologically in the squirrel monkey (Nudo et al., 1996). Considering the patchy, 'clustering' (Huntley and Jones, 1991), 'intermingled' (Schneider et al., 2002), 'colony' (Capaday et al., 1998) forming nature of the M1 cortex, it seems plausible that cortical integration of muscle representation may, to some extent, be a product of use. That increased use, for example a more active lifestyle, would lead to a patchier cortex with greater overlap of muscle representations.

One can speculate that a consequence of 'use dependent' plasticity within M1 would result in increased overlapping of the muscle map with increasing use. Shifts in those subjects with greater cortical integration (early efficient) and overlapping representations may find that such a locus has equal probability of lying in many directions from the initial, mean site of activity. On the other hand, subjects with less cortical integration (late efficient) would have less overlapping of representation of muscles within MI. Hence, loci with a capacity for efficient mediation of new synergies are more probable to lie physically towards the second (deltoid) representation. This could constitute an explanation for our data in terms of differences between groups in the direction of shifts post-training. A second testable consequence of increasing cortical integration of muscle representations due to daily activity was made. As daily physical activity increases, required shifts towards regions most efficient in mediating muscle synergies should become shorter. The post hoc questionnaire applied to test this idea revealed a significant (p < 0.05) negative correlation of distance shifted by the APB representation to daily activity as measured by MET scores. Greater cortical integration would mean a wider area of muscle representation, thus making the representation possibly less prone to complete ablation by focal lesions. This could stimulate the hypothesis that victims with previously active lifestyles should have a better motor recovery of stroke.

Clearly, it must be acknowledged that describing APB or deltoid representations as definitive and exclusive is simplistic. Flexing these muscles independently of other muscles is not possible, BOLD peak points represent performance of the task movement and inherent co-activation of other muscles. However, imaging studies indicate that although there is gross overlapping of motor representations, peak points of dominance retain muscle-related somatotopy (Lotze et al., 2000; Kleinschmidt et al., 1997; Park et al., 2001). We can therefore be sure that the peak point is a good indicator of the muscle's dominant cortical position (see also Gonçalves and Hall, 2003).

Functional connectivity analysis of muscle representations within MI over the short distances described is now plausible. This type of analysis offers a window into how muscle synergies are induced, modulated and maintained. Whether the process of establishing functional connectivity is 'Hebbian', as has been previously implied (Bütefisch et al., 2004) is still open to debate. This question, using the approach presented, now becomes a matter than can be studied *in vivo* in the human with appropriate pharmacological studies. A second fascinating question is how these small dynamics of shifting, possibly competing representations may interact over time in a manner that can account for the gross M1 maps of space and goal directed movements as uncovered previously (Graziano et al., 2002a; Graziano et al., 2002b). Altogether, the method employed here provides a new window into the dynamics of the learning motor system.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2007.01.009.

#### References

- Buechel, C., Coull, J.T., Friston, K.J., 1999. The predictive value of changes in effective connectivity for human learning. Science 283, 1538–1541.
- Bütefisch, C.M., Khurana, V., Kopylev, L., Cohen, G.L., 2004. Enhancing encoding of a motor memory in the primary motor cortex by cortical stimulation. J. Neurophysiol. 91, 2110–2116.
- Capaday, C., 2004. The integrated nature of motor cortical function. Neuroscientist 3, 207–220.
- Capaday, C., Devanne, H., Bertrand, L., Lavoie, B.A., 1998. Intracortical connections between motor cortical zones controlling antagonistic muscles in the cat: a combined anatomical and physiological study. Exp. Brain Res. 120, 223–232.
- Classen, J., Liepert, J., Wise, S.P., Hallett, M., Cohen, L.G., 1998. Rapid plasticity of human cortical movement representation induced by practice. J. Neurophysiol. 79 (2), 1117–1123.
- d'Avella, A., Saltiel, P., Bizzi, E., 2003. Combinations of muscle synergies in the construction of natural motor behaviour. Nat. Neurosci. 6, 300–308.
- Devanne, H., Cohen, L.C., Kouchtir-Devanne, N., Capaday, C., 2002. Integrated motor cortical control of task-related muscles during pointing in humans. J. Neurophysiol. 87, 3006–3017.
- Devanne, H., Cassim, F., Ethier, C., Brizzi, L., Thevenon, A., Capaday, C., 2006. The comparable size and overlapping nature of upper limb distal and proximal muscle representations in the human motor cortex. Eur. J. Neurol. 23, 2467–2476.
- Donoghue, J.P., Sanes, J.N., 1988. Organization of adult motor cortex representation patterns following neonatal forelimb nerve injury in rats. J. Neurosci. 8 (9), 3221–3232.
- Donoghue, J.P., Suner, S., Sanes, J.N., 1990. Dynamic organization of primary motor cortex to target muscles in adult rats: I. Long-term patterns of reorganization following motor or mixed peripheral nerve lesions. Exp. Brain Res. 79 (3), 479–491.
- Donoghue, J.P., Leibovic, S., Sanes, J.N., 1992. Organization of the forelimb area in squirrel monkey motor cortex: representation of digit, wrist and elbow muscles. Exp. Brain Res. 89, 1–19.
- Friston, K.J., Buechel, C., Fink, G.R., Morris, J., Rolls, E., Dolan, R.J., 1997. Psychophysiological and modulatory interactions in neuroimaging. NeuroImage 6 (3), 218–229.
- Gerstein, G.L., Perkel, D.H., 1969. Simultaneously recorded trains of action potentials: analysis and functional interpretation. Science 164, 828–830.
- Gitelman, D.R., Penny, W.D., Ashburner, J., Friston, K.J., 2003. Modeling regional and psychophysiologic interactions in fMRI: the importance of hemodynamic deconvolution. NeuroImage 19 (1), 200–207.
- Gonçalves, M.S., Hall, D.A., 2003. Connectivity analysis with structural equation modelling: an example of the effects of voxel selection. NeuroImage 20, 1455–1467.
- Gould Jr., H., Cusick, C.G., Pons, T.P., Kaas, J.H., 1986. The relationship of corpus callosum connections to electrical stimulation maps of motor, supplementary motor, and the frontal eye fields in owl monkeys. J. Comp. Neurol. 247, 297–325.
- Graziano, M.S.A., Taylor, C.S.R., Moore, T., 2002a. Complex movements evoked by microstimulation of precentral cortex. Neuron 34, 841–951.
- Graziano, M.S.A., Taylor, C.S.R., Moore, T., Cooke, D.F., 2002b. The cortical control of movement revisited. Neuron 36, 349–362.
- Holderfer, R.N., Miller, L.E., 2002. Primary motor cortical neurons encode functional muscle synergies. Exp. Brain Res. 146 (2), 233–243.
- Huntley, G.W., Jones, E.G., 1991. Relation of intrinsic connections to forelimb movement representations in monkey motor cortex: a correlative anatomic and physiological study. J. Neurophysiol. 66, 390–413.
- Jacobs, K.M., Donoghue, J.P., 1991. Reshaping the cortical motor map by unmasking latent intracortical connections. Science 251, 944–947.
- Kelly, A.M.C., Garavan, H., 2005. Human functional neuroimaging of brain changes associated with practice. Cereb. Cortex 15 (8), 1089–1102.
- Kleinschmidt, A., Nitschke, M.F., Frahm, J., 1997. Somatotopy in the

human motor cortex hand area. A high-resolution functional MRI study. Eur. J. Neurosci. 9 (10), 2178–2186.

- Kwan, H.C., MacKay, W.A., Murphy, J.T., Wong, Y.C., 1978. Spatial organization of precentral cortex in awake primates: II. Motor outputs. J. Neurophysiol. 41, 1120–1131.
- Liepert, J., Terborg, C., Weiller, C., 1999. Motor plasticity induced by synchronized thumb and foot movements. Exp. Brain Res. 125 (4), 435–439.
- Lotze, M., Erb, M., Flor, H., Huelsmann, E., Godde, B., Grodd, W., 2000. fMRI evaluation of somatotopic representation in human primary motor cortex. NeuroImage 11, 473–481.
- Nudo, R.J., Jenkins, W.M., Merzenich, M.M., 1990. Repetitive microstimulation alters the cortical representation of movements in adult rats. Somatosens. Motor Res. 7, 463–483.
- Nudo, R.J., Milliken, G.W., Jenkins, W.M., Merzenich, M.M., 1996. Usedependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. J. Neurosci. 16 (2), 785–807.
- Park, M.C., Belhaj-Saif, A., Gordon, M., Cheney, P.D., 2001. Consistent features in the forelimb representation of primary motor cortex in rhesus macaques. J. Neurosci. 21 (8), 2784–2792.
- Pascual-Leone, A., Cammarota, A., Wassermann, E.M., Brasil-Neto, J.P., Cohen, L.G., Hallett, M., 1993. Modulation of motor cortical outputs to the reading hand of braille readers. Ann. Neurol. 34, 33–37.
- Pleger, B., Schwenkreis, P., Dinse, H.R., Ragert, P., Hoffken, O., Malin, J.P., Tegenthoff, M., 2003. Pharmacological suppression of plastic changes in human primary somatosensory cortex after motor learning. Exp. Brain Res. 148, 525–532.
- Sanes, J.N., Donoghue, J.P., 2000. Plasticity and the primary motor cortex. Annu. Rev. Neurosci. 23, 393–415.
- Schneider, C., Devanne, H., Lavoie, B.A., Capaday, C., 2002. Neural mechanisms involved in the functional linking of motor cortical points. Exp. Brain Res. 146, 86–94.
- Sessle, B.J., Wiesendanger, M., 1982. Structural and functional definition of the motor cortex in the monkey (*Macaca fascicularis*). J. Physiol. 323, 245–265.
- Stefan, K., Kunesch, E., Cohen, L.G., Benecke, R., Classen, J., 2002. Mechanism of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J. Physiol. 543 (Pt 2), 699–708.
- Stefan, K., Wycislo, M., Gentner, R., Schramm, A., Naumann, M., Reiners, K., Classen, J., 2005. Temporal occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb. Cortex 16 (3), 376–385.
- Sun, F.T., Miller, L.M., Rao, A.A., D'Esposito, M., in press. Functional connectivity of cortical networks involved in bimanual motor sequence learning. Cereb. Cortex [Electronic publication ahead of print].
- Tegenthoff, M., Witscher, K., Schweinkreis, P., Liepert, J., 1999. Pharmacological modulation of training induced plastic changes in human motor cortex. Electroencephalogr. Clin. Neurophysiol., Suppl. 51, 188–196.
- Tyč, F., Boyadjian, A., Devanne, H., 2005. Motor cortex plasticity induced by extensive training revealed by transcranial magnetic stimulation in human. Eur. J. Neurosci. 21 (1), 259–266.
- Wolbers, T., Schoell, E.D., Verleger, R., Kraft, S., McNamara, A., Jaskowski, P., Büchel, C., 2006. Changes in connectivity profiles as a mechanism for strategic control over interfering subliminal information. Cereb. Cortex 16 (6), 857–864.
- Worsely, K.J., Friston, K.J., 1995. Analysis of fMRI time-series revisitedagain. NeuroImage 2, 173–181.
- Ziemann, U., 2004. TMS induced plasticity in human cortex. Rev. Neurosci. 15 (4), 253–266.
- Ziemann, U., Muellbacher, W., Hallett, M., Cohen, L.G., 2001. Modulation of practice-dependent plasticity in human motor cortex. Brain 124, 1171–1181.
- Ziemann, U., Ilic, T.V., Pauli, C., Meintzschel, F., Ruge, D., 2004. Learning modifies subsequent induction of long-term potentiation-like and longterm depression-like plasticity in human motor cortex. J. Neurosci. 24 (7), 1666–1672.