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Muscle and Timing-specific Functional Connectivity between the Dorsolateral Prefrontal Cortex and the Primary Motor Cortex

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Abstract

■ The pFC has a crucial role in cognitive control, executive function, and sensory processing. Functional imaging, neurophysiological, and animal studies provide evidence for a functional connectivity between the dorsolateral pFC (DLPFC) and the primary motor cortex (M1) during free choice but not instructed choice selection tasks. In this study, twin coil, neuronavigated TMS was used to examine the precise timing of the functional interaction between human left DLPFC and ipsilateral M1 during the execution of a free/specified choice selection task involving the digits of the right hand. In a thumb muscle that was not involved in the task, a conditioning pulse to the left DLPFC enhanced the excitability of the ipsilateral M1 during free selection more than specified selection 100 msec after presentation of the cue; the opposite effect was seen at 75 msec. However, the difference between free and externally specified conditions disappeared when a task-specific muscle was investigated. In this case, the influence from DLPFC was dominated by task involvement rather than mode of selection, suggesting that other processes related to movement execution were also operating. Finally, we show that the effects were spatially specific because they were absent when an adjacent area of DLPFC was stimulated. These results reveal temporally and spatially selective interactions between BA 46 and M1 that are both task and muscle specific. ■

INTRODUCTION

The pFC is highly developed in primates (Miller & Cohen, 2001) and plays important roles in cognitive control, executive function, working memory, and top–down modulation of sensory processing (Miller & Cohen, 2001; Miller, 2000). Within pFC, the dorsolateral pFC (DLPFC) has a central integrative function for motor control and behavior. In particular, Brodmann's area 46 (BA 46) has diverse neuronal connections to several different motor regions such as the premotor cortices, SMA, cerebellum, and BG (Miller & Cohen, 2001; Lu, Preston, & Strick, 1994; Bates & Goldman-Rakic, 1993; Alexander, DeLong, & Strick, 1986). Animal studies involving monkeys indicate that the lateral pFC in particular plays a crucial and superordinate role in motor selection decisions for adapting two behavioral rules (Hoshi, Shima, & Tanji, 2000). In humans, imaging studies have shown that activation of the DLPFC (especially BA 46) is prominent during action selection, particularly in tasks in which participants are required to freely select their movement (Rowe, Stephan, Friston, Frackowiak, & Passingham, 2005; Hadland, Rushworth, Passingham, Jahanshahi, & Rothwell, 2001; Hoshi et al., 2000; Jueptner et al., 1997; Deiber, Ibanez, Sadato, & Hallett, 1996; Spatt & Goldenberg, 1993; Deiber et al., 1991; Frith, Friston, Liddle, & Frackowiak, 1991). For example, one early study in which rCBF was measured with PET, showed increased activation of the DLPFC when participants made free selection responses relative to when they were specified (Frith et al., 1991). Another PET study showed that free selection conditions activated various cortical areas, including different motor cortical fields, but that there was an exclusive increase of rCBF in pFC compared with the activation pattern following cued conditions. The authors concluded that the internal selection process for self-selection of movements involves a distributed network located mainly in the frontal lobe (Deiber et al., 1996). Later fMRI studies confirmed these ideas and showed that the coupling between DLPFC and M1 is greater for freely selected choices compared with external instructed choices (Rowe et al., 2005). Finally, work using TMS has revealed a distinct inhibitory network involving two frontal brain regions, the lateral pFC and the dorsal premotor cortex (PMd), and the interconnected M1 during response preparation of selected and unselected effectors (Duque, Labruna, Verset, Olivier, & Ivry, 2012; Duque, Lew, Mazzocchio, Olivier, & Ivry, 2010). This work suggests that during freely selected movements, specific

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interactions exist between the DLPFC (especially BA 46) and M1. However, little is known regarding the exact timing and the excitatory and inhibitory nature of this DLPFC–M1 interaction during action selection tasks. Therefore, the present experiments were designed to probe the details of a specific interaction between DLPFC and M1, using twin coil TMS.

In this design, one coil is used to stimulate M1 to probe the excitability of corticospinal output to hand muscles involved in the task; the other is used to stimulate BA 46 at 6–12 msec beforehand. There are no direct anatomical connections between DLPFC and M1 (Miller & Cohen, 2001), but TMS connectivity studies indicate a coupling between pFC and M1 at subsecond timescales. For example, one TMS study investigated the connections between M1 and frontal/medial cortices at rest and showed an inhibitory influence of premotor stimulation on the M1 at short ISIs (4–6 msec; Civardi, Cantello, Asselman, & Rothwell, 2001). Some of the positions of the conditioning coil used in those experiments (6 cm anterior to the hot spot) could be considered as overlapping with the area defined as DLPFC (Rusjan et al., 2010; Fitzgerald, Maller, Hoy, Thomson, & Daskalakis, 2009). However, the translation from this pioneering work to cognitive neuroscience is not simple, as no neuronavigation was used and connectivity was examined at rest rather than during the execution of a task as in this study. As noted by others, connectivity between brain areas is often quite different in different behavioral states (Rothwell, 2011).

By varying the time of stimulation after a cue, which signalled either a free selection or specified finger movement, we assessed whether the interactions between BA 46 and M1 occurred at particular intervals during task preparation and if this was specific to free selection. In addition, because EMG activity evoked by M1 stimulation can be recorded in separate hand muscles we also ask whether the influence of BA 46 is specific to muscles involved in the task. Finally, we used neuronavigation to position the site of DLPFC stimulation. Therefore, we could investigate whether the interaction was spatially specific to BA 46 by applying the conditioning stimulus to the rostral part of the superior frontal gyrus (BA 9), which is also considered part of the DLPFC (Petrides & Pandya, 1999).

We tested the hypothesis that the excitability of the functional connection between a given region of DLPFC, namely BA 46, and the ipsilateral motor cortex is modulated during a choice reaction task. In our model, modulation would depend on the modality of the task, the timing of the cue presentation, the selection/nonselection of an effector and the localisation of pFC stimulation.

METHODS

Participants

Seventeen participants (10 women, mean age = 30.2 ± 7.0 years) participated in one or more of the experiments of this study. Ten participants (eight women) participated in Experiment 1, seven participants (three women) participated in Experiment 2, and Experiments 3 and 4 were each conducted with eight participants (four women). For all experiments, participants had individual T1-weighted MRI scans. All participants were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971) and had normal or corrected-to-normal vision. There was no history of neurological or mental illness, alcohol or drug abuse, metallic cerebral implants, and no participant was taking any neuroactive medication. The study protocol, which is in accordance with the Declaration of Helsinki, was approved by the Ethics Committee of University College London.

Behavioral Task

Participants performed an instructed free selection/external specified selection task similar to that described in previous publications (Rowe et al., 2005). Participants sat in front of a standard computer screen, which was approximately 80 cm, in front of them. In brief, a white arrow was presented every 5 sec in the middle of a black screen. In the externally specified condition, the arrow could occur at four different orientations (9, 11, 1 and 3 o'clock) each of which specified a button press of a different finger (respectively: index finger, middle finger, ring finger, small finger). A fifth arrow with an orientation at 12 o'clock indicated that this was a free selection trial in which participants had to select at will any finger press. To avoid perseveration, in the free selection trials participants were instructed not to repeatedly use the same finger but to make a random choice on each occasion (Rowe et al., 2005). Participants performed one practice block with 30 trials before the experiment started. In Experiment 1, 960 trials were applied in four blocks (240 trials/block, 120 free selection and 120 specified selection). In Experiments 2, 3, and 4, 480 trials were applied in four blocks (120 trials/block, 60 free selection and 60 specified selection). After each block, a pause of approximately 7 min was given. Each 24 trials were fully randomized; therefore, neither the participant nor the experimenter could predict the trial order.

TMS

We recorded surface EMG from the right abductor pollicis brevis muscle (APB, Experiments 1 and 3) and the right first dorsal interosseous muscle (FDI, Experiments 1, 2, and 4) via Ag/AgCl electrodes in a belly-tendon montage. Raw signals were amplified (Digitimer 360, Digitimer Ltd., Welwyn Garden City, Herts, UK), band-pass filtered (10 Hz–3 kHz) and digitalized using a 1401 data acquisition interface (Cambridge Electronic Design Ltd., Cambridge, UK) controlled by Signal Software (Cambridge Electronic Design). To investigate the BA 46–M1 connectivity within

the left hemisphere, two figure-of-eight coils (7 cm outer diameter for the primary motor cortex (M1), 5 cm outer diameter for the BA 46 region) connected to two single-pulse monophasic stimulators (Magstim Co., Whitland, Dyfeld, UK) were used. With this experimental design, the influence of DLPFC on M1 could be quantified by measuring the extent to which DLPFC stimulation changed the excitability of the ipsilateral M1 outputs. In contrast to most other TMS connectivity studies, we investigated pFC (in our study BA 46)–M1 connection within the same hemisphere. This was achievable through the use of a small custom-made figure-of-eight coil and in the selection of an area that was located at a sufficient distance to M1 to allow a reliable placement of two figure-of-eight coils on the same hemisphere. This setup reduced the bias derived from interhemispheric measures and allowed us to focus on the dominant hemisphere. The intensity of the conditioning pulse (BA 46) was set at 105% of resting motor threshold (RMT) and the intensity of the test pulse (M1) was set to evoke a 1-mV motor-evoked potential (MEP) at rest with the large TMS coil. The decision to set the intensity of the conditioning pulse at 105% RMT was based on the findings that a suprathreshold conditioning pulse can elicit functional interactions between the frontal lobe and M1 (O'Shea, Sebastian, Boorman, Johansen-Berg, & Rushworth, 2007; Koch et al., 2006) and on the observation that higher stimulation intensities used over this area were less well tolerated by our participants. RMT was defined as the lowest intensity that produced an MEP of >50 μV in 5 of 10 trials in the relaxed target muscle with the small TMS-coil placed over the left M1. The left M1 was defined functionally as the position where single-pulse TMS induced consistently the largest MEPs in both reference muscles (Figure 1).

Individual anatomical T1 MRI scans and Brainsight Neuronavigation (Rogue Research, Canada) was used to determine the exact location of the left BA 46 site (Talairach coordinates $[x, y, z]$: -40, 28, 30) previously linked to the specification of freely selected actions (Rowe et al., 2005). This position was visually inspected and corrected when necessary by A.H. to ensure a target position on the gray matter. Talairach coordinates (Talairach & Tournoux, 1998) were transformed into native space using the brain atlas function Brainsight Neuronavigation (Rogue Research, Canada).

Experimental Design

During all experiments, participants were placed in front of a screen and wore a tight-fitting EEG cap with the marked TMS coil positions.

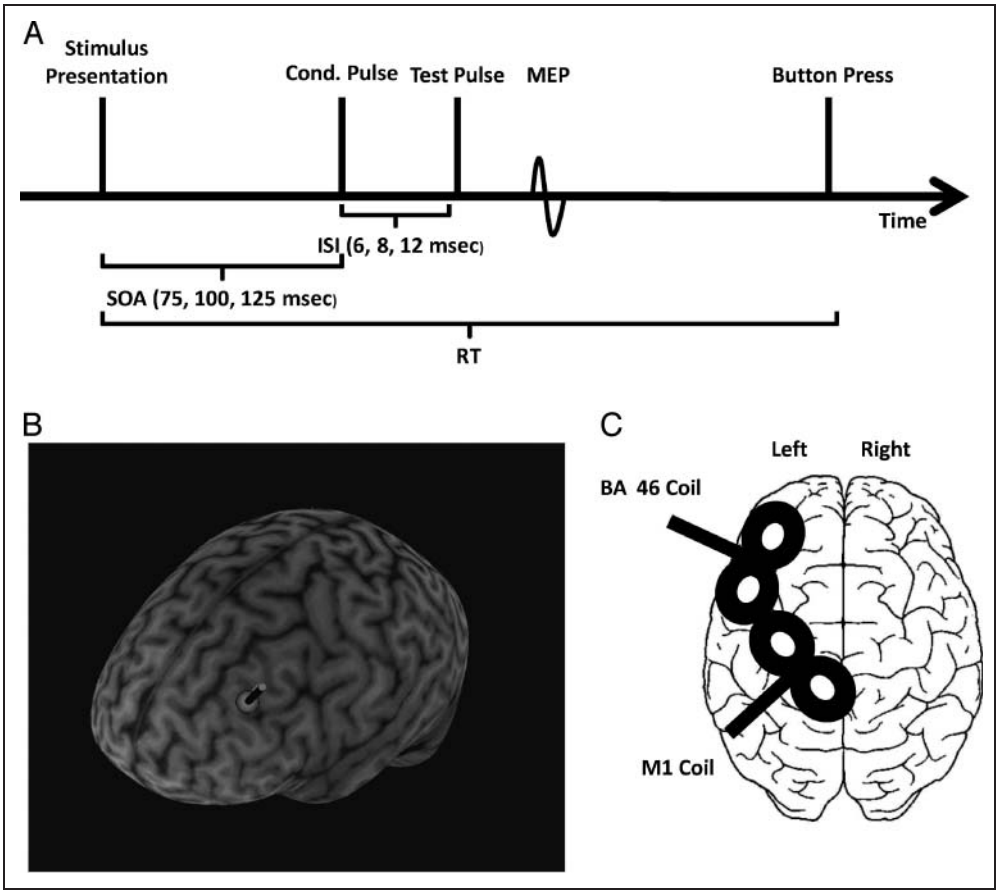

Figure 1. (A) Time course of the BA 46–M1 experiment. The conditioning pulse was applied 75, 100, or 125 msec after the cue appeared on the screen. The test pulse followed this conditioning pulse with a latency of 6, 8, or 12 msec in Experiment 1. (B) Stimulation site and coil placement of the BA 46 coil (3-D reconstructed brain images of one representative participant). (C) Schematic presentation of the coil placements over the left hemisphere.

Experiment 1: Influence of BA 46 Stimulation on the Excitability of the Ipsilateral M1 Measured in an Unselected Muscle

This experiment tested the effect of stimulation of BA 46 on the excitability of corticospinal output from M1 to a muscle that was not involved in any of the four possible finger movements (APB) and to an involved muscle (FDI). In the first experiment, we used an unselected muscle as primary outcome measure for two reasons: first, we wanted to avoid any possible effect of movement preparation on corticospinal excitability of M1, which is expected in task related muscles. Second, the findings of Rowe et al. (2005) from their fMRI study indicated that DLPFC was exerting a nonsomatotopic effect on M1 suggesting that it would be apparent in all muscles of the involved hand. However, to test our hypothesis of this non-specific connectivity, we analyzed the data of the FDI as a secondary outcome measure and compared the results from both muscles in this experiment.

Three different SOAs between the appearance of an arrow on the visual display and the conditioning TMS pulse were examined (SOA; 75, 100, 125 msec) at three different ISIs between stimulation of BA 46 and M1 (ISI; 6, 8, 12 msec). These SOAs and ISIs were based on those used to investigate the connectivity of premotor/frontal brain regions and the M1 within and between hemispheres (Buch, Mars, Boorman, & Rushworth, 2010; Neubert, Mars, Buch, Olivier, & Rushworth, 2010; Mars et al., 2009; O'Shea et al., 2007; Koch et al., 2006).

Experiment 2: Specific and Muscle-dependent BA 46–M1 Connectivity

Experiment 2 tested the effect of BA 46 stimulation on corticospinal excitability to selected and unselected muscles at different SOAs (75, 100, 125 msec) and a single ISI (12 msec), which was identified as optimal from Experiment 1. A single ISI was chosen so that we could record a sufficient number of trials for each finger response to allow a comparison between selected and unselected muscles during free and specified trial types. In these trials, RTs and EMG data from movements with an index finger press (FDI selected) were contrasted with data from movements in which the correct finger press was middle, ring, or small finger (FDI not selected).

Experiments 3 and 4: Anatomic Specificity of the BA 46–M1 Connectivity

Anatomical specificity was tested in two additional control experiments. Experiments 3 and 4 were similar to Experiments 1 and 2 (uninvolved and involved muscles, respectively), except that the conditioning coil was placed over BA 9 rather than BA 46 (x, y, z: −9, 50, 21, BA 9 region).

Presentation of visual stimuli and synchronization with TMS was implemented by MATLAB 2008b (The MathWorks, Natick, MA) and the Cogent toolbox developed by LON, FIL, and ICN at University College London (www.vislab.ucl.ac.uk/cogent.php).

Assessing Randomness of Free Choices

Although participants were instructed to choose a response randomly within free selection, it is possible that certain patterns would emerge (Jahanshahi, Dirnberger, Fuller, & Frith, 2000; Robertson, Hazlewood, & Rawson, 1996). We compared the level of randomness within free and specified trials. To this end, we calculated the entropy conveyed by trials (Harrison, Duggins, & Friston, 2006). Trial-by-trial entropy (H) was calculated as

$$H(x) = -\sum f(x) * \log 2 f(x)$$

where x (1 of the 16 possible combinations between finger selected on trial t and trial $t-1$) is a discrete random variable and $f(x)$ is the value of its probability distribution at x. Entropy was estimated separately for the free selection and specified trial types within Experiment 2 (this experiment had a large number of homogenous trials to allow such a post hoc analyses) and compared across participants with a paired t test.

Data Analysis/Statistical Analyses

To correct for small differences in coil placement and possible alterations in baseline MEPs and SOAs between blocks, MEP sizes were normalized within each block and analysis was performed across blocks. RTs were defined from the onset of the cue until the button press and analyzed as absolute values. Trials with incorrect responses precontraction in the target muscle (EMG amplitude in 100 msec before the TMS pulse $> 2.5 \times$ EMG amplitude 800–1000 msec before the TMS pulse) or RTs less than 80 msec were excluded from further analyses. RTs were analyzed as absolute values to allow the assessment of single-pulse and paired-pulse TMS on RTs.

For statistical analyses, SPSS 20 for Windows was used. Level of significance was set at $\alpha = .05$. Shapiro–Wilk tests confirmed normal distribution for the data ($p > .05$). Electrophysiological data (MEP-Amplitude) and behavioral data (RT-Duration) were analyzed with repeated-measures ANOVAs (RM-ANOVA) in a within-subject design. If appropriate (significant interactions in the RM-ANOVA), Student's t tests (paired sample or one-sample, two-tailed) were performed to determine changes between different conditions and in comparison with the baseline. In the linear

models, sphericity was tested with Mauchly's test, and if necessary (Mauchly's test < 0.05), the Greenhouse–Geisser correction was used.

RESULTS

Assessing Randomness of Free Choices

The following post hoc analysis was conducted on all participants in Experiment 2. A paired t test showed no significant difference ($t(6) = 1.451$, $p = .197$) in entropy between the conditions (free: 1.74 ± 0.07 bits, specified: 1.78 ± 0.05 bits). This indicates that the degree of randomness of finger selection was similar across free and specified trial types. Additionally, in the free selection condition, Finger 1 was chosen in 26.0% (±9.2%), Finger 2 in 27.1% (±3.1%), Finger 3 in 29.5% (±9.1%), and Finger 4 in 18.0% (±4.8%). RM-ANOVA with the factor Finger showed no significant difference in the distribution of fingers used within the free trial types, $F(1.3, 7.7) = 3.141$, $p = .112$.

Correct and Incorrect Trials

In Experiment 1, 4.6% of the trials in the free selection condition and 6.4% of the trials in the externally cued condition were incorrect. Experiment 2 had 2.9% incorrect free and 5.3% incorrect specified trials. The control Experiments 3 and 4 respectively had 5.4% and 5.2% incorrect free selected trials and 6.9% and 6.7% incorrect specified selected trials. All incorrect trials were excluded from the analysis.

Experiment 1: BA 46–M1 Connectivity in an Unselected Muscle (APB, Nonspecific Connectivity)

Behavioral Data

One participant had to be excluded from the analysis because she did not complete all blocks. The three-way RM-ANOVA (RT absolute values) with the factors Condition (free selection vs. specified selection), SOA (75, 100, 125 msec), and TMS (single pulse [test pulse only], 6, 8, 12 msec) revealed a significant main effect of Condition, $F(1, 8) = 22.539$, $p = .01$, indicating, as expected, faster RTs in the specified selection trials. Furthermore, analyses revealed a significant main effect of SOA, $F(2, 16) = 9.789$, $p = .01$, but no further main effects or interactions (all $F < 1.849$, $p > .110$) (Table 1).

Electrophysiological Data

The aim of Experiment 1 was to investigate whether the influence of BA 46 on corticospinal excitability of M1 (assessed at ISIs of 6, 8, 12 msec) changed at different times after the appearance of the visual go signal (SOAs of 75, 100, 125 msec). As detailed below, the results indicate that during trials with externally specified responses, stimulation of BA 46 increases excitability of M1 at a SOA of 75 msec but does not modulate it at SOAs of 100 and 125 msec. However, in freely selected trials, stimulation of BA 46 at a SOA of 100 msec facilitates M1 excitability. Averaged data suggested that these effects occurred at all three ISIs, but additional analyses show that the main effect is at an ISI of 12 msec.

To compare the MEPs recorded from both muscles, we used a four-way ANOVA with the factors Muscle (APB vs.

Table 1. RTs for Experiment 1

Specified Selection				*Free Selection*				
Pulse	*SOA*	*Mean (msec)*	*SD*	*Pulse*	*SOA*	*Mean (msec)*	*SD*	*p*
Test pulse	75	620.9	35.1	Test pulse	75	714.6	54.4	**.0004**
PP 6 msec	75	636.3	55.5	PP 6 msec	75	697.0	64.5	**.023**
PP 8 msec	75	655.0	56.0	PP 8 msec	75	714.6	61.0	**.020**
PP 12 msec	75	635.4	55.0	PP 12 msec	75	712.7	53.3	**.007**
Test pulse	100	651.5	51.2	Test pulse	100	718.1	57.6	**.002**
PP 6 msec	100	660.1	60.1	PP 6 msec	100	731.9	57.0	**.002**
PP 8 msec	100	661.2	59.0	PP 8 msec	100	724.8	60.5	**.006**
PP 12 msec	100	650.0	59.0	PP 12 msec	100	736.1	58.4	**.002**
Test pulse	125	676.3	56.6	Test pulse	125	726.6	85.9	**.032**
PP 6 msec	125	647.7	68.4	PP 6 msec	125	723.6	50.8	**.002**
PP 8 msec	125	654.0	59.0	PP 8 msec	125	729.0	63.7	**.003**
PP 12 msec	125	660.8	58.5	PP 12 msec	125	737.0	73.9	**.009**

Data in **bold**: $p < .05$ (comparison specified selection vs. free selection, paired t tests, two-tailed). PP = paired-pulse.

FDI), Condition (free selection vs. specified selection), SOA (75, 100, 125 msec), and ISI (6, 8, 12 msec). This analysis revealed a trend for a Muscle × Condition × SOA interaction, $F(2, 16) = 2.824$, $p = .089$, and a trend for a Condition × ISI interaction, $F(2, 16) = 2.931$, $p = .082$, but not further main effects or interactions (all Fs < 2.544, $p > .111$).

For the APB muscle (unselected muscle, nonspecific connectivity), we performed a three-way RM-ANOVA with factors Condition (free vs. specified), SOA (75, 100, 125 msec), and ISI (6, 8, 12 msec). This revealed a significant Condition × SOA interaction, $F(2, 16) = 6.674$, $p = .008$, a trend for an interaction Condition × ISI, $F(2, 16) = 2.773$, $p = .092$, but no further main effects or interactions (all Fs < 1.386, $p > .279$). To enhance the power of this analysis by reducing the input to the ANOVA, ISIs were merged together as one factor Mean ISI. As expected from the results of the first ANOVA, this analysis revealed a significant Condition × SOA interaction, $F(2, 16) = 6.163$, $p = .010$, but no further main effects of interactions (all $F < 0.835$, $p > .453$). These Mean ISI values were used for contrasting the Condition × SOA interaction.

Paired-sample t tests showed a significantly higher paired-pulse/single-pulse ratio for free selection (1.12 ± 0.09) compared with specified selection (0.97 ± 0.15) at a SOA of 100 msec ($t(8) = 3.138$, $p = .014$) and a lower paired-pulse/single-pulse ratio for free selection (1.02 ± 0.16) compared with specified selection (1.19 ± 0.25) at a SOA of 75 msec ($t(8) = 2.312$, $p = .050$; Figure 2A). One-sample t tests of the ratios against baseline (test value = 1.00; Neubert et al., 2010) showed that the MEPs were significantly facilitated at a SOA of 100 msec in the free selection condition ($t(8) = 4.063$, $p = .004$) and that MEPs showed a trend toward facilitation at a SOA of 125 msec ($t(8) = 2.067$, $p = .072$). In the specified condition, MEPs showed a trend toward a facilitation at a SOA of 75 msec ($t(8) = 2.283$, $p = .052$; Figure 2A).

These results could not be confirmed in the FDI muscle. The three-way ANOVA with factors Condition (free vs. specified), SOA (75, 100, 125 msec), and ISI (6, 8, 12 msec) did not show, apart from a trend for an interaction Condition × ISI, $F(2, 16) = 2.921$, $p = .083$, any main effects or interactions (all $F < 0.913$, $p > .423$).

The interaction between PMd/PMv/SMA and the ipsilateral and contralateral M1 was found to be within 10 msec at rest and during the performance of various behavioral tasks (Buch et al., 2010; Baumer et al., 2009; Davare, Lemon, & Olivier, 2008; Baumer et al., 2006; Koch et al., 2006; Mochizuki, Huang, & Rothwell, 2004). Therefore, we can assume that the interaction, which is very likely to be polysynaptic, between BA 46 and ipsilateral M1 should be in the range of ISIs longer than 10 msec. For that reason, we hypothesized that our observed effect would be greatest at an ISI of 12 msec, and although we had no effect of the factor ISI in the initial ANOVA, we repeated our analyses with this ISI (12 msec) to confirm

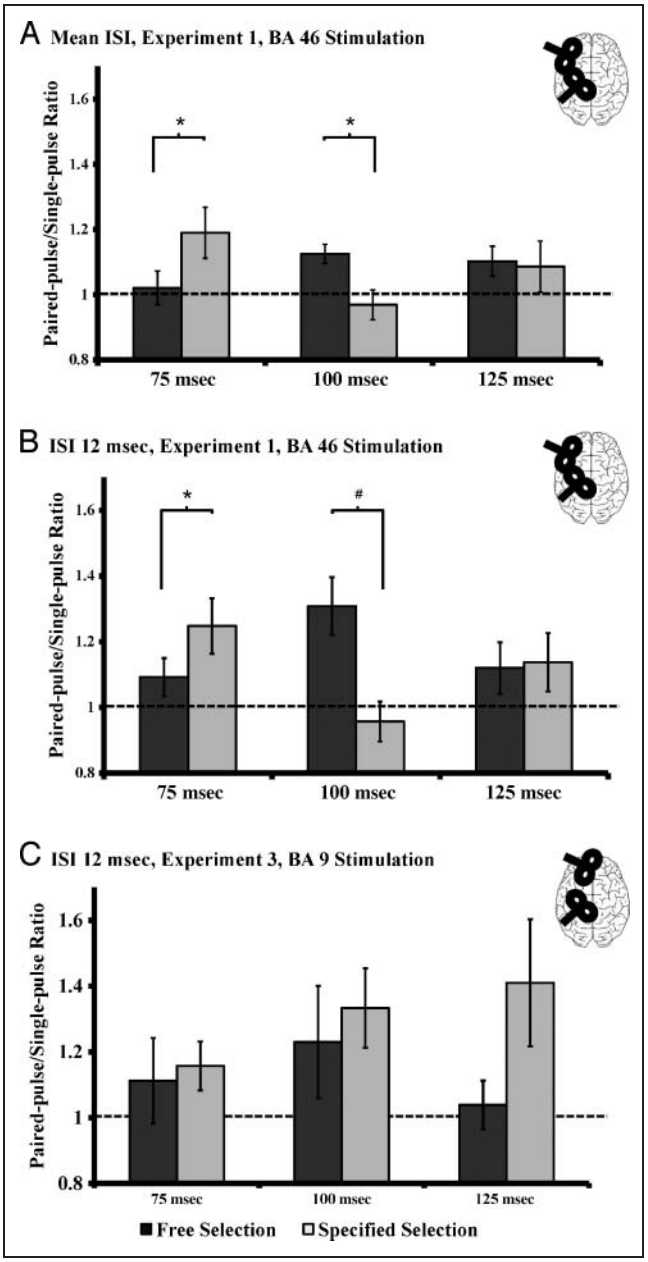

Figure 2. Timing of the non-specific functional connectivity (data recorded from the right APB, Experiment 1 [cross-interaction] and Experiment 3). (A) BA 46 stimulation: Paired-pulse/single-pulse ratio averaged for all ISIs (6, 8, 12 msec) at different SOAs (75, 100, 125 msec). At a SOA of 75 msec, the functional BA 46–M1 connectivity is enhanced for trials with an external specified action, and at a SOA of 100 msec, the functional BA 46–M1 connectivity is enhanced for free selection trials. This indicates different timings of stimulus processing in the visual and frontal lobe. (B) BA 46 stimulation: Paired-pulse/single-pulse ratio for one ISI (12 msec) at different SOAs (75, 100, 125 msec). This shows that the main effect is driven by an ISI of 12 msec, and for that reason, all further experiments were conducted using an ISI of 12 msec. (C) BA 9 stimulation: Paired-pulse/single-pulse ratio for one ISI (12 msec) at different SOAs (75, 100, 125 msec). The cross-interaction at the SOAs of 75 and 100 msec disappeared, and analyses could not detect an effect of BA 9 stimulation on M1 excitability. The visual difference at a SOA of 125 msec is because of an outlier and not statistically significant. $^{*}p \leq .05$, $^{\#}p < .08$ (trend level). Error bars are expressed as *SEM*.

our initial findings, which were calculated with the factor Mean ISI.

To compare both muscles, we used a three-way ANOVA with the factors Muscle (APB vs. FDI), Condition (free selection vs. specified selection), and SOA (75, 100, 125 msec). This analysis revealed a trend for a significant Muscle × Condition × SOA interaction, $F(2, 16) = 2.951$, $p = .083$, but no further main effects or interactions (all Fs < 0.633, $p > .545$).

For the APB muscle, RM-ANOVA with RM-ANOVA with the factors Conditions and SOA revealed a significant Condition × SOA interaction, $F(2, 16) = 7.327$, $p = .006$, but no main effects (all Fs < 1.328, $p > .283$). Paired-sample t tests showed a significantly higher paired-pulse/single-pulse ratio for free selection (1.31 ± 0.26) compared with specified selection (0.96 ± 0.18) at a SOA of 100 msec ($t(8) = 3.692$, $p = .006$) and a trend for a lower paired-pulse/single-pulse ratio for free selection (1.09 ± 0.17) compared with specified selection (1.25 ± 0.25) at a SOA of 75 msec ($t(8) = 2.036$, $p = .076$; Figure 2B). One-sample t tests of the ratios against baseline (test value = 1.00) showed that the MEPs were significantly facilitated at a SOA of 100 msec in the free selection condition ($t(8) = 3.500$, $p = .008$) and that MEPs were facilitated in the specified selection condition at a SOA of 75 msec ($t(8) = 2.932$, $p = .019$; Figure 2B).

For the FDI muscle, RM-ANOVA with the factors Conditions and SOA revealed no Condition × SOA interaction, $F(2, 16) = 0.169$, $p = .846$, and no main effects (all Fs < 0.258, all ps $> .627$).

Baseline cortical excitability. To examine possible changes in baseline cortical excitability, we performed a statistical comparison of the single-pulse TMS trials. RM-ANOVA for the APB with the factors Condition and SOA revealed no main effects (all Fs < 3.125, all ps $> .112$), but a significant Condition × SOA interaction, $F(2, 16) = 4.369$, $p = .030$. Post hoc paired t tests indicate that the baseline MEP amplitudes were smaller in the free selection condition (0.42 ± 0.30 mV) compared with the specified selection condition (0.52 ± 0.38 mV) at a SOA of 100 msec ($t(8) = 3.332$, $p = .011$). At a SOA of 75 msec (free: 0.47 ± 0.40 mV, specified: 0.43 ± 0.32 mV) and a SOA of 125 msec (free: 0.45 ± 0.34 mV, specified: 0.45 ± 0.35 mV), post hoc t tests showed no differences of baseline MEPs.

For the FDI, RM-ANOVA revealed no main effects for Condition, $F(1, 8) = 0.733$, $p = .417$, or SOA, $F(2, 16) = 0.343$, $p = .715$, and no Condition × SOA interaction, $F(2, 16) = 1.002$, $p = .389$. There were no differences in baseline MEPs at a SOA of 75 msec (free: 1.41 ± 1.07 mV, specified: 1.35 ± 1.03 mV), a SOA of 100 msec (free: 1.42 ± 1.10 mV, specified: 1.47 ± 1.19 mV), or a SOA of 125 msec (free: 1.43 ± 1.14 mV, specified: 1.35 ± 0.97 mV).

In summary, these results indicate an interaction between BA 46 and M1 at SOAs of 75 and 100 msec, which is dependent on task modality. However, we cannot determine the precise ISI of this interaction. On the basis of our literature-based hypothesis that a longer ISI most likely underlies this interaction and the additional analyses focussing on an ISI of 12 msec, we decided to use only one ISI, namely 12 msec, for the following experiments. This allowed us to accumulate more trials for the involved and noninvolved muscles.

Experiment 2: BA 46–M1 Connectivity in a Selected Muscle (FDI, Muscle-specific Connectivity)

Behavioral Data

This experiment, conducted in seven participants (three women) was similar to Experiment 1, except that in this case we examined corticospinal excitability to a muscle involved in the task (FDI: index finger press). A four-way RM-ANOVA (RT absolute values) with the factors Condition (free vs. specified), SOA (75, 100, 125 msec), Selection (selected vs. not selected), and TMS (single pulse vs. 12 msec) revealed an expected significant main effect of Condition, $F(1, 6) = 10.881$, $p = .016$, a significant Condition × Selection interaction, $F(1, 6) = 12.010$, $p = .013$, a trend for a significant Condition × SOA interaction, $F(3, 12) = 3.157$, $p = .079$, but no further main effects or interactions (all Fs < 2.408, $p > .173$). RTs for this experiment and further contrasts are presented in Table 2. In general, as in Experiment 1, RTs were faster in the specified condition than the freely selected condition.

Electrophysiological Data

We separated out trials into those in which the movement was an index finger press (FDI involved) and movements of any of the other three fingers (FDI not involved). In contrast to Experiment 1, there was no difference between freely selected and externally instructed movements (Figure 3A). The main result was that BA 46–M1 connectivity was facilitated in trials in which an index finger press was to be made, but there was no effect in trials where a different finger was moved. This was confirmed using RM-ANOVA with the factors Condition (free vs. specified), SOA (75, 100, 125 msec), and Selection (selected vs. not selected). This revealed a significant main effect of Selection, $F(1, 6) = 22.516$, $p = .003$, but no further main effects or interactions (all Fs < 3.30, $p > .120$; Figure 3).

Baseline cortical excitability. RM-ANOVA with the factors Condition, Selection, and SOA revealed a significant Condition × Selection interaction, $F(1, 6) = 6.312$, $p = .046$, but no main effects (all Fs < 1.720, $p > .238$), and no further interactions (all Fs < 0.992, $p > .399$). Post hoc t tests showed a higher MEP baseline for not-selected trials compared with selected trials at a SOA of 125 msec ($t(6) = 2.472$, $p = .048$; selected: 0.71 ± 0.51 mV,

Table 2. RTs for Experiments 2 and 4

Specified Selection				*Free Selection*				
Pulse	*SOA*	*Mean (msec)*	*SD*	*Pulse*	*SOA*	*Mean (msec)*	*SD*	*p*
Experiment 2								
Selected								
Test pulse	75	615.9	124.4	Test pulse	75	731.2	126.0	**.043**
PP 12 msec	75	642.6	117.9	PP 12 msec	75	747.6	187.7	.098
Test pulse	100	763.0	250.6	Test pulse	100	784.6	206.5	.388
PP 12 msec	100	647.8	119.0	PP 12 msec	100	747.5	187.7	**.031**
Test pulse	125	633.6	95.5	Test pulse	125	809.0	151.1	**.001**
PP 12 msec	125	641.0	108.0	PP 12 msec	125	774.0	194.0	**.026**
Not selected								
Test pulse	75	656.9	124.8	Test pulse	75	691.9	184.4	.307
PP 12 msec	75	663.7	124.0	PP 12 msec	75	694.4	170.4	.304
Test pulse	100	687.8	158.0	Test pulse	100	650.4	327.0	**.011**
PP 12 msec	100	679.3	132.5	PP 12 msec	100	753.6	188.0	.723
Test pulse	125	689.5	131.2	Test pulse	125	713.5	153.6	.216
PP 12 msec	125	682.5	118.9	PP 12 msec	125	762.1	157.9	**.004**
Experiment 4								
Selected								
Test pulse	75	661.6	110.9	Test pulse	75	682.5	99.0	.609
PP 12 msec	75	648.7	72.6	PP 12 msec	75	703.5	97.5	.117
Test pulse	100	665.7	90.0	Test pulse	100	668.8	131.0	.901
PP 12 msec	100	672.6	79.0	PP 12 msec	100	727.7	99.4	**.018**
Test pulse	125	691.0	104.1	Test pulse	125	758.0	102.8	.226
PP 12 msec	125	664.4	76.3	PP 12 msec	125	758.4	94.3	**.019**
Not selected								
Test pulse	75	647.3	84.1	Test pulse	75	682.4	93.7	.237
PP 12 msec	75	617.0	60.4	PP 12 msec	75	663.3	111.9	.111
Test pulse	100	661.1	87.7	Test pulse	100	684.6	124.7	.278
PP 12 msec	100	655.1	94.6	PP 12 msec	100	714.7	108.7	**.037**
Test pulse	125	668.3	62.4	Test pulse	125	702.8	135.1	.502
PP 12 msec	125	656.4	74.3	PP 12 msec	125	688.5	95.4	.108

Data in **bold**: $p < .05$ (comparison specified selection vs. free selection, paired t tests, two-tailed). PP = paired-pulse.

not-selected: 1.00 ± 0.58 mV) during the execution of an instructed task. In the free selection task, the baseline MEPs at a SOA of 75 msec were larger in selected trials (1.24 ± 0.86 mV) compared with not-selected trials (0.79 ± 0.52 mV; $t(6) = 2.484$, $p = .048$). Comparing both conditions, MEPs in a selected muscle were marginally larger in freely selected trials at a SOA of 125 msec compared with specified trials ($t(6) = 2.402$, $p = .053$; free: 1.00 ± 0.78 mV; specified: 0.71 ± 0.51 mV). In an unselected muscle, MEPs were marginally smaller in freely selected trials at a SOA of 75 msec ($t(6) = -2.297$, $p = .053$; free: 0.80 ± 0.52 mV; specified: 1.00 ± 0.70 mV). No other contrasts showed significant results (all $t < 1.521$, $p > .179$).

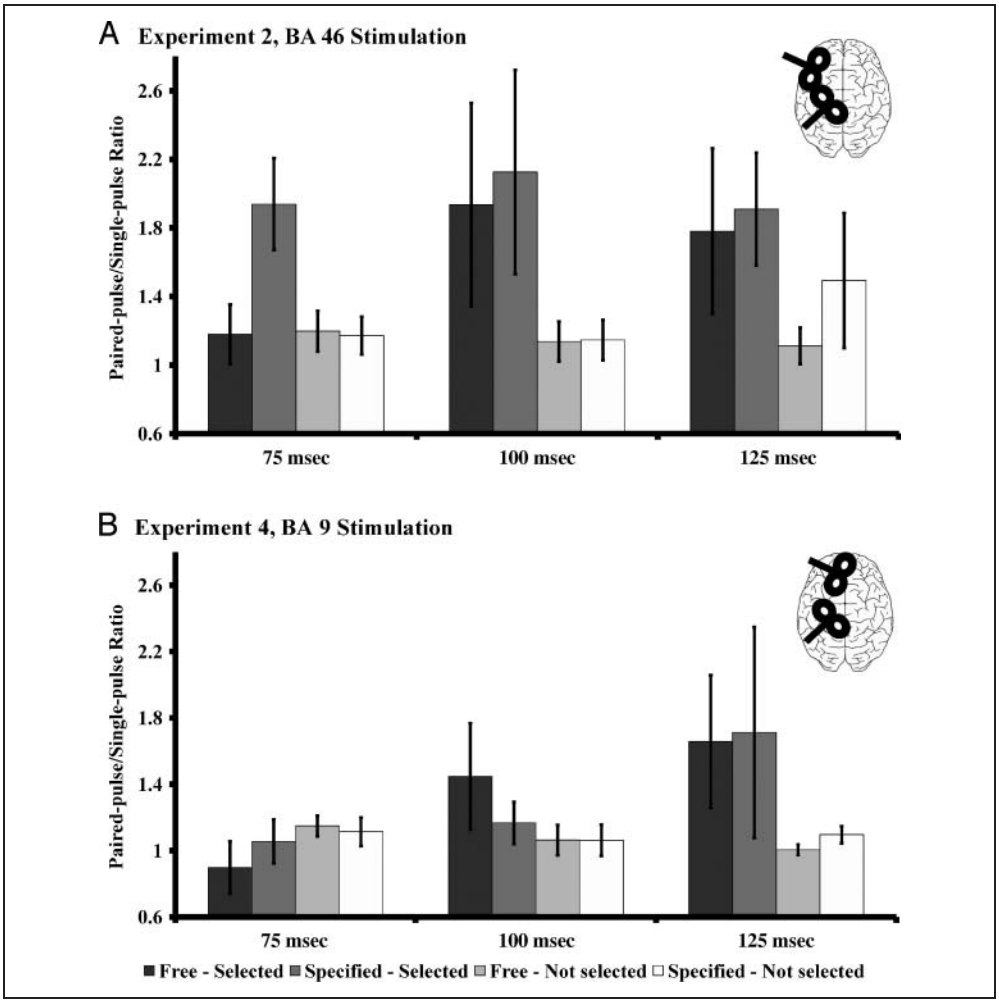

Figure 3. Timing of the specific functional connectivity with regard to muscle involvement (data recorded from the right FDI, Experiments 2 and 4). (A) BA 46 stimulation: The difference between free and externally specified conditions disappeared (compare with Figure 2, Experiment 1) when a task-specific muscle was investigated (significant main effect of "selection"). Different relay stations from BA 46, such as premotor cortices, might influence BA 46–M1 connectivity when task-specific, selected muscles are investigated. (B) BA 46 stimulation: Analyses did not reveal an effect of BA 9 stimulation and the initially described effect (A) disappeared. The visual difference at a SOA of 125 msec is because of an outlier and not statistically significant. The data of 75 and 100 msec shows clearly that BA 46 stimulation has an impact on selected movements and the stimulation of BA 9 is not able to replicate this finding. Error bars are expressed as *SEM*.

Experiment 3: Control Experiment for the Nonspecific BA 46–M1 Connectivity (Anatomical Specificity): BA 9–M1 Connectivity in an Unselected Muscle (APB, Nonspecific Connectivity)

Behavioral Data

Eight participants (four women) participated in this experiment. One participant did not have enough valid recordings and was excluded. A three-way RM-ANOVA (RT absolute values) with the factors Condition (free selection vs. specified selection), SOA (75, 100, 125 msec), and TMS (single pulse [test pulse only], 12 msec) revealed a significant main effect of SOA, $F(2, 12) = 5.761$, $p = .018$, a trend for Condition, $F(1, 6) = 5.192$, $p = .063$, a trend for TMS, $F(1, 6) = 5.623$, $p = .055$, but no interactions ($F < 1.781$, $p > .211$; Table 3).

Table 3. RTs for Experiment 3

Specified Selection				*Free Selection*				
Pulse	*SOA*	*Mean (msec)*	*SD*	*Pulse*	*SOA*	*Mean (msec)*	*SD*	*p*
Test pulse	75	645.3	82.1	Test pulse	75	661.5	89.5	**.042**
PP 12 msec	75	620.2	63.5	PP 12 msec	75	658.9	97.5	**.004**
Test pulse	100	647.6	66.1	Test pulse	100	663.1	109.6	**.003**
PP 12 msec	100	641.5	80.4	PP 12 msec	100	667.9	106.3	**.001**
Test pulse	125	644.2	65.9	Test pulse	125	705.9	111.9	.118
PP 12 msec	125	652.0	69.3	PP 12 msec	125	693.0	84.9	**.014**

Data in **bold**: $p < .05$ (comparison specified selection vs. free selection, paired t tests, two-tailed). PP = paired-pulse.

Electrophysiological Data

RM-ANOVA with the factors Condition (free vs. specified) and SOA (75, 100, 125 msec) did not reveal any main effect or interactions (all Fs < 2.136, $p > .194$) showing that the observed connectivity (Experiment 1) is critically dependent on BA 46 and not on a general frontal lobe activation (Figure 2C).

Experiment 4: Control Experiment for the Muscle-specific BA 46–M1 Connectivity (Anatomical Specificity): BA 9–M1 Connectivity in a Selected Muscle (FDI, Muscle-specific Connectivity)

Behavioral Data

Eight participants (four women) participated in this experiment. Two participants did not have enough valid recordings and were excluded. A four-way RM-ANOVA (RT absolute values) with the factors Condition (free vs. specified), SOA (75, 100, 125 msec), Selection (selected vs. not selected), and TMS (single pulse vs. 12 msec) revealed a trend for a main effect of Condition, $F(1, 5) = 5.047$, $p = .075$, a significant effect of SOA, $F(2, 10) = 5.236$, $p = .028$, but no further main effects (all Fs < 1.858, $p > .232$). Apart from a Condition × TMS interaction, $F(1, 5) = 7.739$, $p = .042$, no other interactions could be detected (all Fs < 2.044, $p > .213$; Table 2).

Electrophysiological Data

RM-ANOVA with the factors Condition (free vs. specified), SOA (75, 100, 125 msec), and Selection (selected vs. not selected) did not reveal any main effect or interactions (all Fs < 1.387, $p > .292$). In accordance with the findings of Experiment 3, the muscle-specific connectivity found in Experiment 2 is dependent on stimulation of BA 46 (Figure 3).

DISCUSSION

The present results reveal temporally and spatially selective interactions between BA 46 and M1 that are both task and muscle specific. The latency of the effects was short and occurred with stimulation of BA 46 only 6, 8, or 12 msec prior to M1. Although additional analyses suggested that the main effect occurred at the longest ISI of 12 msec, the data are in line with the idea that BA 46 has an intimate influence on motor cortical excitability. However, whether later effects also occur is unknown, as we did not investigate longer ISIs. Because there are no direct connections between BA 46 and M1, likely candidates might involve a relay in PMd or other secondary cortical motor areas (Miller, 2000; Lu et al., 1994; Luppino, Matelli, Camarda, & Rizzolatti, 1993; Strick, 1985). An anatomically direct pathway between premotor and primary motor cortex can be activated at ISIs of 4–6 msec (Civardi et al., 2001; Godschalk, Mitz, van Duin, & van der Burg, 1995). Subcortical pathways through the BG might also contribute (Neubert et al., 2010; Miller & Cohen, 2001; Alexander et al., 1986), although this is perhaps more likely at the longer intervals given the correspondingly longer pathways and multiple relays that would be involved.

Stimulation of BA 46 Has a Bidirectional and Timing-specific Effect on Motor Cortical Excitability during the Execution of a Choice Selection Task

Experiment 1 showed that, during a free selection task, stimulation of BA 46 facilitated M1 excitability. This effect was maximal at 100 msec after the instruction cue, occurred in a muscle controlling a digit (thumb) that was not involved in the task itself (finger pressing), and was not seen if the movement was instructed rather than freely selected. At the earlier SOA (75 msec), stimulation of BA 46 facilitated M1 to a greater extent during instructed movement than during free selection in this noninvolved muscle. This facilitation was greater than at baseline. The first implication of these findings is that visual information about the instruction signal rapidly reaches prefrontal areas. This signal is processed within pFC, and dependent on the timing of the stimulus presentation and the modality of the stimulus, the connectivity to the motor system is modulated. When this action signal indicates that participants must freely choose their next finger movement, it increases the excitability of facilitatory interactions between BA 46 and muscle representations in M1 whereas this connectivity is significantly inhibited if the cue specifies the required movement. On the other hand, the early timing of the facilitatory interaction at a SOA of 75 msec following a cue for an instructed movement may indicate that this information is evaluated more quickly than free choice. Because it was facilitatory, it could contribute to the shorter RTs to externally instructed compared with freely selected movements. In summary, the excitability of the BA 46–M1 interaction varied with the mode of selection and the time point of the task.

One previous fMRI study using a related task design found greater activation of dorsal pFC (especially BA 46) and M1 in the free selection condition, whereas both conditions resulted in activation of the prefrontal lobe. Furthermore, there was significantly greater coupling between left BA 46 and M1 in the free selection of the task (Rowe et al., 2005). The results of our experiments provide additional evidence about the task-related timing of BA 46–M1 interactions, but further studies focussing on disrupting possible cortical relay areas (e.g., with repetitive TMS protocols) are needed to clarify the precise functional anatomy of this connection. We suggest that a facilitatory influence of BA 46 on M1 excitability at a SOA of 100 msec may contribute to the increased functional coupling between these two cortical areas observed during

free selection tasks in fMRI studies (Rowe et al., 2005). Furthermore, our findings indicate the BA 46–M1 connection can be facilitated when pFC is processing external instructed movements at earlier timings, a finding that has not been presented before.

The role of the DLPFC in free selection tasks has been well established by fMRI, PET, and TMS studies (Hadland et al., 2001; Jueptner et al., 1997; Deiber et al., 1991, 1996; Frith et al., 1991) and is reinforced by our findings. During the free selection process, we propose that the DLPFC sends a facilitatory and specific output to ipsilateral M1. However, it should be noted that, although the DLPFC is associated with action selection, it may not be involved in action execution (Bunge, Hazeltine, Scanlon, Rosen, & Gabrieli, 2002). This role in selection but not generation of a specific movement may explain why we could observe an influence of DLPFC on corticospinal outputs to a muscle that was not involved in the task itself.

At a SOA of 100 msec, we observed higher single-pulse MEP amplitudes in cued conditions and a facilitation of paired-pulse MEP amplitudes for free conditions. Studies using paired-pulse paradigms applied to the primary motor cortex (e.g., short-interval intracortical inhibition or intracortical facilitation) indicate that the inhibitory or facilitatory effect is related to the sizes of test MEPs (Chen, 2004). Therefore, a direct intra-area effect within the left M1 could be one possible additional explanation of the observed excitability shift.

The Impact of the Ipsilateral DLPFC Is Reduced in Muscles Involved in the Choice Reaction Task

Unlike Experiment 1, the modality of movement selection (Experiment 2) had no effect on the excitability of muscles involved in the task. M1 output to these muscles was influenced by whether or not the muscle was used in the upcoming movement. Thus, a muscle involved in index finger flexion was facilitated from BA 46 whenever participants had to press the response button with their index finger but was unaffected when a different finger was used. This occurred whether the movement was chosen freely or specified by the instruction cue. Therefore, involvement of a muscle in the task had a stronger influence on BA 46–M1 connectivity than the free and specified conditions. Indeed, the magnitude of the effect was much larger in task-related muscles than in those that were never used. In addition, the effects when the muscle was not selected were the same in the specified and free conditions and did not change with time, unlike the effects we saw in the non-involved muscle in Experiment 1. It is possible that the input from BA 46 to M1 interacts with other inputs that either excites or suppresses task relevant muscles. These other inputs may mask the smaller effects observed in uninvolved muscles within Experiment 1. This is unlikely to occur within M1 itself because facilitation from BA 46 is expressed relative to the ongoing level of excitability in M1. Given that the anatomical BA 46–M1 connection is necessarily indirect, the observed interaction may well occur at an intermediate stage(s) of the pathway.

One possibility is that the effects during the specified trials are relayed via PMd. Duque et al. (2012) recently showed that stimulation of the contralateral PMd during the presentation of a preparatory cue in a choice reaction task facilitated motor cortical output to an involved (effector) muscle but had no effect in a nonselected muscle (Duque et al., 2012). In contrast, stimulation of the contralateral lateral prefrontal region reduced inhibition in both selected and not-selected effectors, suggesting that the lateral pFC is responsible for general and abstract aspects of motor control (Duque et al., 2012). Note, however, that our results are based on ipsilateral BA 46–M1 connectivity, whereas the effect observed by Duque et al. (2012) represents an interhemispheric connectivity.

Other data confirm that the intermediate relay stations from BA 46, such as premotor areas, influence activation in muscles involved in the task and at similar timings to BA 46 (Miller & Cohen, 2001). For example, Koch and colleagues (2006) showed that PMd modulates activation in muscle groups involved in a task while having no effect on muscles that are uninvolved. In choice reaction tasks, like that in the present experiments, it facilitated muscles when they were selected in the task but suppressed them when they were not selected. Despite some differences in experimental paradigms, it could be that similar effects occur even in freely chosen movements, with facilitation of the chosen muscle and suppression of any potential candidate muscles. Indeed, input from BA 46 during free selection trials could act as an appropriate trigger for such behavior, which may dominate the influence of BA 46 on M1 that are described in Experiment 1.

In this hypothetical framework, we can assume that the results of Experiment 1 reflect a relatively "pure" influence of BA 46 on M1, which we observe as changes in excitability of non-involved muscles during free selection, whereas the results of Experiment 2 might represent a cumulative effect of different inhibitory and facilitatory inputs to M1.

The Effect of the DLPFC on Motor Cortical Excitability Is Anatomically Specific and Dependent on the Stimulation of BA 46

It is important to note that, within the frontal lobe, different subregions have unique functions in cognitive control, as well as interconnections that fulfill their biological function (Miller, 2000). The DLFPC is occupied by the interconnected cytoarchitectonic areas BA 9 and BA 46 (Petrides & Pandya, 1999), and the findings of our study indicate that, during a selection task with a motor response (finger press), the functional connectivity from one part of the DLPFC, namely BA 46, to M1 is of particular importance. In our additional experiments, we found no connectivity between BA 9 and M1 using the experimental configuration, which showed a prominent effect

after stimulating BA 46. It should be noted that determination of exact anatomical borders of BA 9 and BA 46 is difficult (Petrides & Pandya, 1999) and that other SOAs, ISIs, or another task design might be necessary to probe the BA 9–M1 connection. However, our findings underlie the importance of subdividing the DLPFC according to function.

Conclusions

The present results suggest that there is anatomically specific functional connectivity between left BA 46 and left M1 during free and specified selection of a movement. In selected muscles, the input of the DLPFC has only limited impact on the M1 excitability, as other more powerful inputs from various areas of the motor network may modulate M1 excitability. A direct functional connection between DLPFC and M1, as suggested by imaging studies, seems to have a minor role in this complex network and is only unmasked in uninvolved muscles. Our results provide further evidence for a functional specialization within the DLPFC and reveal that connectivity changes at specific time intervals during a choice reaction task.

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