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The motor cortical representation of a muscle is not homogeneous in brain connectivity

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ABSTRACT

Functional connectivity patterns of the motor cortical representational area of single muscles have not been extensively mapped in humans, particularly for the axial musculature. Functional connectivity may provide a neural substrate for adaptation of muscle activity in axial muscles that have both voluntary and postural functions. The purpose of this study was to combine brain stimulation and neuroimaging to both map the cortical representation of the external oblique (EO) in primary motor cortex (M1) and supplementary motor area (SMA), and to establish the resting-state functional connectivity associated with this representation. Motor evoked potentials were elicited from the EO muscle in stimulation locations encompassing M1 and SMA. The coordinates of locations with the largest motor evoked potentials were confirmed with task-based fMRI imaging during EO activation. The M1 and SMA components of the EO representation demonstrated significantly different resting-state functional connectivity with other brain regions: the SMA representation of the EO muscle was significantly more connected to the putamen and cerebellum, and the M1 representation of the EO muscle was significantly more connected to somatosensory cortex and the superior parietal lobule. This study confirms the representation of a human axial muscle in M1 and SMA, and demonstrates for the first time that different parts of the cortical representation of a human axial muscle have resting-state functional connectivity with distinct brain regions. Future studies can use the brain regions of interest we have identified here to test the association between resting-state functional connectivity and control of the axial muscles

KEYWORDS

Functional connectivity, primary motor cortex, supplementary motor area, transcranial magnetic stimulation

Muscles have specific mechanical actions that they perform on the body. They are activated by a diffuse network of neurons distributed across motor cortical regions including the primary motor cortex (M1), cingulate motor area, and supplementary motor area (SMA) (Boudrias et al. 2010; Kakei et al. 1999). Mapping the connectivity of the portions of these distinct motor cortical regions representing the same muscle may offer important clues about how that muscle is controlled, for example, how it is activated appropriately under varying task constraints. This may be particularly true of muscles with diverse task-specific roles. For example, the human abdominal musculature demonstrates distinct patterns of activation during forced expiratory efforts, postural alterations, and voluntary movements (Chiou et al. 2016; Kumar 2004; Moseley et al. 2004; Tunstill et al. 2001). Within motor cortex, SMA is often associated with preparation of complex movements involving postural adjustments (Viallet et al. 1981; Tanji 1994) as well as voluntary respiratory effort (McKay et al. 2003), while M1 is associated with voluntary motor execution (Stippich et al. 2007; Solodkin et al. 2004). Therefore, investigation of the human abdominal musculature representation in M1 and SMA and the connectivity associated with this representation may provide unique insight into the neural correlates of task-specific activation of an individual muscle.

The broadly somatotopic organization of muscle and movement representation in M1 is well established (Penfield and Boldrey 1937). Intracranial stimulation studies have demonstrated some somatotopic organization of SMA in animals and humans (Mitz and Wise 1987; Fried et al., 1991) but evidence to date has focused on limb representation. In humans, non-invasive mapping studies utilizing transcranial magnetic stimulation (TMS) have suggested that motor cortical representation of axial musculature including the paraspinals and diaphragm extends anteriorly into SMA (O'Connell et al. 2007; Sharshar et al. 2004). However, without confirmation from neuroimaging, it is unclear if these findings truly reflect SMA representation of trunk musculature or rather are an artifact due to current spread, the distance between the

stimulating coil and the motor area, or lack of selectivity of the surface electrodes utilized to measure evoked potentials (Thickbroom et al. 1998). It is also unclear whether each region has a separate representational area for a given muscle, or whether there is a a single muscle representational area that encompasses contiguous regions of both SMA and M1.

Functional connectivity, as quantified with resting-state fMRI, maps both direct and indirect connections within functional brain networks (Buckner et al. 2013; Damoiseaux and Greicius 2009; Grafton et al. 2016). To our knowledge, existing investigations of the functional connectivity between human motor cortex and other cortical and sub-cortical regions have exclusively focused on seed regions in the lateral motor cortex, identified from upper limb motion (Biswal et al. 1995; Guye et al. 2003). It is already clear that axial musculature has more excitable ipsilateral corticospinal output than limb musculature (Strutton et al. 2004), and that axial and appendicular motor control is associated with different pathways in the basal ganglia (e.g. Visser et al. 2008). Therefore, it should not be assumed that the connectivity of the axial musculature of the medial motor cortex with the rest of the brain is identical to that of the limb musculature. Resting-state functional connectivity has recently been shown to predict inter-individual differences in brain activity during task performance during hand muscle use (Tavor et al. 2016). If the resting-state functional connectivity patterns associated with axial muscles could be mapped, future studies would be ideally positioned to determine if inter-individual differences in a much wider variety of movements could be predicted from resting-state functional connectivity.

The purpose of this study was to take the initial steps in this line of research by identifying the motor cortical representation of an axial muscle and then mapping the whole-brain functional connectivity of this representation. First, we aimed to establish coordinates for the cortical representational area of the external oblique muscle in MNI stereotactic space utilizing single-pulse TMS, and to confirm these coordinates utilizing task-based fMRI. Second, we aimed to determine the location of this representation relative to the different anatomical

sub-divisions (Brodmann areas) of motor cortex. We hypothesized that the external oblique representation would encompass both the primary motor cortex and posterior supplementary motor area. Finally, we probed the resting-state functional connectivity of the muscle representation and the rest of the brain.

MATERIALS AND METHODS

TMS acquisition and analysis.

TMS Participants

Thirteen healthy young adults (eight women, five men; mean age: 25.8 ± 2.1 years) participated in the study. Individuals were eligible to take part in the study if they were between 18 and 30 years old. The upper age limit was utilized to avoid the potential confounding influence of age on brain structure and function (~~Kong et al. 2013~~Ferreira & Busatto, 2013; Seidler et al. 2010). Exclusion criteria were a history of significant musculoskeletal disorders, including back or hip pain, as well as neurological disorders and contraindications to receiving TMS. *A priori* power analysis indicated that eleven participants would be sufficient to quantify muscle representation coordinates with a power of at least 80%. All participants provided informed consent and the study procedures were approved by the Institutional Review Board of the University of Southern California.

TMS procedures

Each participant was fitted with a Lycra cap with 1 cm grid markings, and disposable self-adhesive electromyography (EMG) electrodes were placed over the contralateral external oblique muscle (EO) (inter-electrode distance 22mm; Myotronics-Noromed, Inc, Kent, WA). The electrode was placed slightly anterior to the midpoint of the distance between the iliac crest and lower lateral border of the rib cage (Vera-Garcia et al. 2010). EMG data were bandpass filtered between 10 and 1000Hz and amplified at a gain of x2000 (Motion Lab Systems, Baton Rouge,

LA). Single-pulse stimuli were delivered by a 110mm double cone coil (MagStim 200^2, the MagStim Company Ltd, MagStim, UK). EMG data and digital output from the TMS unit were sampled into the computer at 15000Hz. This high sampling frequency was utilized in order to be able to visualize a TTL output from the Magstim unit indicating when the TMS pulse was delivered during data collection and post-processing. Starting with the coil placed 2 cm lateral and anterior to the vertex (Strutton et al. 2004), the optimal site of stimulation or "hotspot" of the EO was determined by delivering a series of pulses around this area to find the scalp location that induced the largest and most consistent motor-evoked potential (MEP).

Following the identification of the EO "hotspot", the active motor threshold (AMT) was quantified by determining the minimum stimulation intensity to evoke five consecutive MEPs that were distinguishable from the background EMG activity during a sub-maximal active contraction. For later consistency of coil placement during data collection, an infra-red marker tracking system (Brainsight®, Rogue Research Inc., Montreal, Canada) was used to co-register the subject's scalp anatomical landmarks to a 3D representation of a standard brain magnetic resonance image scan. This also allowed us to estimate stimulation locations in standard MNI coordinates. Prior to data collection, maximum voluntary isometric contraction (MVIC) of EO was determined by applying manual resistance to the shoulders as the individual performed maximal trunk flexion/rotation in the supine position. For data collection, five TMS pulses were delivered at 120% AMT at each point on a 6 x 4 cm grid that included the hotspot (120 total pulses) while the subject contracted the EO to 20% MVIC (O'Connell et al. 2007; Tsao et al. 2011;Schabrun et al. 2017). To ensure a consistent level of muscle activation, the subjects utilized real-time visual biofeedback to achieve 20% (+/- 5%) MVIC. Subjects rested in a supine position for 5-10 seconds between each TMS stimulus. Neuronavigation confirmed that the 6 x 4 cm grid used during data collection encompassed the pre-central gyrus including both supplementary motor area (SMA) and primary motor area (M1).

TMS data processing and analysis

MEP data were acquired and processed with Signal software (Cambridge Electronic Design Limited, Cambridge, UK) and then exported to MATLAB® for further analysis (MathWorks, Natick, USA). Background EMG activation in a 100 ms window prior to the TMS pulse onset was calculated with a root mean square average. MEP amplitude was calculated as the peak-to-peak amplitude of each MEP with the mean background activation subtracted. The average MEP amplitude for the five stimuli at each grid point was then calculated. These average MEP magnitudes for each grid point were normalized with respect to each subject's maximum MEP magnitude. There are multiple ways to quantify the location of the cortical representation of a muscle from MEP magnitude. These include calculation of the amplitude-weighted "center of gravity" for the representation, or determination of the single location that provided the largest MEPs. As we wished to explore the extent of the representation across M1 and SMA, rather than determining a single point, we adopted a two-part approach. Firstly, the existence of separate peaks or foci of representation within the motor map for each individual was explored by determining grid locations where MEP amplitude at a grid location was greater than the amplitude of all of the surrounding grid sites. Secondly, a one-way ANOVA (factor; grid location, with 24 levels) with two-tailed Tukey HSD correction for multiple comparisons was then used to identify grid locations with MEP amplitudes that were significantly greater than other locations.

Task-based fMRI acquisition and analysis.

The TMS study provided mediolateral (x) and rostrocaudal (y) coordinates for grid locations with the largest MEPs in standard MNI space, projected onto the cortex surface. The task-based fMRI study was then conducted to confirm these coordinates and establish the dorsoventral coordinates of EO by determining the location of voxels in the motor cortex that became significantly active during EO muscle contraction.

Participants

A second group of 7 healthy adults (4 female) with a mean age 27.4 ± 3.5 years participated in the task-based fMRI study. Procedures were performed at the University of Southern California and approved by the University of Southern California Institutional Review Board. All participants provided informed consent.

fMRI procedures

Before the experimental session, participants were trained to perform an abdominal bracing maneuver at approximately 20% of MVIC in a mock MRI scanner. During the training, muscle activation in the EO was monitored in real-time with surface EMG and participants were provided with visual and auditory cuing to maintain the correct level of activity until they were able to contract at the correct level without feedback. Participants were also trained to perform the contraction while keeping the head still.

The study utilized a 3 Tesla scanner (GE Signa Excite) with an eight-channel head coil. Subjects were positioned in supine (with a bolster under the knees) while viewing a fixation crosshair, with foam pads within the head coil used to limit head motion. In the scanner, subjects were asked to contract their abdominal muscles to approximately 20% effort in the same way as during the training session. As in our previous fMRI studies of various muscle tasks (Asavasopon et al. 2014; Kutch et al. 2015; Rana et al. 2015) T2-weighted echo planar image volumes with blood oxygen level-dependent (BOLD) contrast (echo time, 34.5 ms; flip angle, 90°; field of view, 220 mm; pixel size, 3.43 mm) were collected continually every 2.5 s during the abdominal run (six 30 second blocks of 15 repeated contractions, each contraction lasting two seconds, interspersed with 30 second blocks of rest). Each volume consisted of 37 axial slices (3 mm slice thickness, 0.5 mm interslice gaps) that covered the brain from vertex to cerebellum. Additionally, a T1-weighted high-resolution anatomical image was acquired from each subject.

fMRI data processing and analysis

Each participant's fMRI data were pre-processed using the FMRIB Expert Analysis Tool (FEAT - http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), which included skull extraction using the brain extraction tool in FSL, slice timing correction, motion correction, spatial smoothing using a Gaussian kernel with full width half maximum (FWHM) of 5 mm, and nonlinear high-pass temporal filtering (100 s). A general linear model (GLM) was used to examine the changes in BOLD signal associated with EO muscle activation. Participant-level whole-brain GLM analyses for each participant were used to determine the change in BOLD signal during the contraction blocks compared with the rest blocks. A group-level mixed-effect (FLAME 1 in FSL) analysis was then performed to identify voxels in standard MNI coordinates with significant increases in BOLD signal associated with EO contraction compared to rest. Group-level images were performed with cluster-based correction for multiple comparisons with $Z > 2.3$ and $p < 0.05$.

Task-based fMRI and TMS to create motor cortical regions-of-interest (ROI).

The thresholded statistical map provided by the task-based fMRI experiment of EO muscle contraction, along with the TMS stimulation grid locations that elicited significant MEPs in the EO muscle, were used to identify voxels that were 1) activated during EO contractions and 2) closer to locations on the TMS stimulation grid that produced significant MEPs than to locations that did not. Inferences about specific Brodmann areas were made using the Jülich Histological Atlas within FSL (Eickhoff et al. 2005). The motor cortical ROI for rs-fMRI functional connectivity analysis were the following: the BA4 ROI (M1) was defined to be all voxels meeting the above criteria (1 & 2) that were most likely BA4. The BA6 ROI (SMA) was defined to be all voxels meeting the above criteria (1 & 2) that were most likely BA6.

rs-fMRI functional connectivity analysis of motor cortical ROI.

Participants

Functional connectivity analyses were performed on a set of resting-state rs-fMRI images from 200 participants from the 1000 Functional Connectome Project (http://www.nitrc.org/ir/). From this repository, two large datasets with 100 participants in each

were constructed - the first dataset was used for discovery and the second was used for validation. Participants were selected according to the following criteria. 1) Participant had 3T rs-fMRI scans with TR=2000 ms, 2) Participant age and sex were available in the repository metadata, 3) Participant was in the age range of the TMS and task-based fMRI studies. Head motion limits were adjusted until 200 participants meeting the criteria with the smallest amount of head motion were selected (selected participants had no more than 0.6 mm of head translation and no more than 1.2° of head rotation). These 200 participants were randomly assigned to the two groups so that each group would have equal numbers of men and women and the groups would be as age-matched as possible. The final group of participants had 122 women and 78 men, with an age of 22.1 ± 2.5 years.

Resting-state fMRI data processing and analysis

Resting-state (rs-) fMRI functional connectivity analyses were performed as GLM analyses in FSL as in previous studies (Rana et al. 2015). Pre-processing of each participant's rs-fMRI time series was performed using FEAT and included skull extraction using the brain extraction tool (BET) from FSL, motion correction, spatial smoothing using a Gaussian kernel of full-width half-maximum of 5 mm and nonlinear high-pass temporal filtering (150 s). The first four volumes were removed to allow for signal stabilization. In each participant, average signals from the BA4 and BA6 ROIs (seed signals) were constructed. The functional connectivity of each of these signals with each voxel in the brain was quantified by a GLM, modeling each voxel's BOLD signal as a combination of either the BA4 signal or the BA6 signal and several sources of noise of no-interest (six parameters obtained by rigid body correction of head motion, the whole-brain signal averaged over all voxels of the brain, a signal from a ventricular ROI, and a signal from a white matter ROI). Functional connectivity for each region was represented as the regression coefficient for the seed signals in the GLM. Individual participant maps of BA4 functional connectivity and BA6 functional connectivity were then registered to standard MNI space for cross-participant statistical analysis.

Validation of the results was performed as follows. In the discovery group, maps of the effect size of BA6 functional connectivity > BA4 functional connectivity across participants were made and examined. Based on inspection of the spatial distribution of effect size in the discovery group, we chose to validate functional connectivity differences in 4 areas: basal ganglia, cerebellum, primary somatosensory cortex, and the superior parietal lobule. Voxels in each of these regions that had effect size magnitudes of at least 0.3 (small but meaningful effect size, Cohen 1998) were identified. Then, and without any further modification, a functional connectivity difference value (functional connectivity with BA6 minus functional connectivity with BA4) was extracted for the identified voxels in the 4 areas (averaging across voxels within each area for each participant) in the separate validation group. A t-test within the validation group was used to confirm that functional connectivity difference values were significantly different from 0 and matched the direction of change observed in the discovery group. As an additional analysis that did not depend on an effect size threshold, within each participant we averaged the functional connectivity difference (BA6-BA4) for all voxels within a small 3mm radius sphere centered at the location of peak effect size in the discovery group for each of the 4 areas described above. We report the coordinates and effect size for the functional connectivity difference in Table 1. The coordinates, without further modification, were used to extract the average functional connectivity differences from all participants in the validation group. These functional connectivity differences were compared to 0 (no difference in functional connectivity between BA6 and BA4) using a t-test, and these validation results are also reported in Table 1.

RESULTS

In all individuals, the grid location with the largest average MEP amplitude was within M1. Eleven out of the thirteen the participants demonstrated MEP amplitudes of at least 60% of their maximum amplitude in grid locations corresponding to SMA. Of these, five had separate peaks in MEP amplitude in SMA and M1. Across the group, eight grid locations produced MEPs

with amplitudes that were significantly greater than at least one other grid location ($F_{(23, 288)}$ = 6.58, $p < 0.0001$; circled grid locations in **Figure 1a**). MEPs from three exemplar points are highlighted in **Figure 1a**). The coordinates of the eight significant locations were retained for further analysis.

Significantly active voxels associated with the EO contraction in the three exemplar points are shown in **Figure 1b**. Regions closest to the eight significant TMS locations were composed of voxels associated with BA6 anteriorly and BA4 posteriorly **(Figure 1c).** These regions became the ROI for seed-based resting state functional connectivity analysis. The BA6 ROI was centered at MNI coordinates (x,y,z) of -8, -18, 70 and had a volume of 1.7 cc (cubic centimeter); the BA4 ROI was centered at -10, -32, 72 and had a volume of 1.1 cc (Table 1).

In the discovery group, the BA6 representation of the EO muscle was significantly more functionally connected to the basal ganglia (putamen) and cerebellum compared to the BA4 representation of this muscle. The BA4 representation of the EO muscle was significantly more functionally connected to somatosensory cortex and the superior parietal lobule compared to the BA6 representation of this muscle (**Figure 2a & b**). These findings were confirmed in the validation group.

DISCUSSION

This study utilized multiple imaging modalities to establish and validate the representation of the human abdominal musculature in M1 and SMA, and to probe the functional connectivity of the sub-regions within this representation. Our study extends previous findings in animal and human studies, and adds to the very limited existing research investigating the functional interaction of a muscle representation with the rest of the brain.

Our work confirms previous findings that suggested that the trunk muscle encompasses SMA as well as in M1 (O'Connell et al. 2007). Although previous TMS studies have mapped the trunk musculature, their use of skull–based landmarks rather than MNI coordinates to report

map locations limited the precise determination of where in the motor cortex trunk muscle representation is located (Schabrun et al. 2015; Tsao et al. 2011; Tsao et al. 2010). Importantly, the combination of our TMS and task-based fMRI results demonstrates for the first time that the size and location of this representational area is not an artifact of TMS methodology.

With a large, age-matched group for the rs-fMRI analyses, we were able to determine that the functional brain connectivity deriving from the motor representation of external oblique varies significantly across sub-regions of the motor cortex. This divergent connectivity closely parallels the distinct functions of this muscle. In addition to its involvement in forced expiratory maneuvers (Ito et al. 2016), the external oblique has a postural function during any activity requiring upright alignment, such as walking (White and McNair 2002), and is a component of anticipatory postural adjustments in association with destabilizing limb motion (Hodges et al. 2003). Its role in goal-directed voluntary motion is evident during gross motions such as trunk rotation, and during the more complex movements required by skilled motor activities such as gymnastics or dance (Kumar 2004; Tunstill et al. 2001).

We demonstrate that the sub-region of the EO representation in SMA has greater resting state connectivity with the putamen and cerebellum than the representation in M1. These findings are consistent with the postural and respiratory functions of external oblique. SMA is activated during preparation and initiation of voluntary movements (Lee et al. 1999; Tanji 1994). In addition to the planning of the voluntary motor actions, neuroimaging and lesion studies have demonstrated that SMA is involved with the timing and amplitude of the anticipatory postural muscle activity that occurs prior to voluntary motor actions (Bolzoni et al. 2015; Ng et al. 2011; Viallet et al. 1992). Activity in the putamen during anticipatory postural adjustments has been demonstrated in healthy adults (Ng et al. 2011) while impairments in anticipatory postural adjustments are evident in individuals with Parkinson's disease (Bazalgett et al. 1987) with known dysfunction of the putamen. An existing study of resting-state functional connectivity of SMA also indicated connectivity between SMA and putamen but utilized an ROI in the pre-SMA

area obtained during task-based fMRI of finger tapping. This area may have greater association with motor preparation and initiation than postural control (Wu et al. 2011; Tanji et al.1994). The resting state functional connectivity that we observed between SMA and the cerebellum is also consistent with a role in postural activity of the external oblique. The area identified in the present study, Lobule VI, forms part of the anterior, motor region of the cerebellum (Stoodley and Schmahmann 2010). It is proposed that the anterior cerebellum contributes to the scaling, temporal organization and adaptability of anticipatory and compensatory postural muscle activity (Diener et al. 1989; Jacobs and Horak 2007; Schmitz et al. 2005).

Primary motor cortex (M1) is believed to encode the intrinsic and extrinsic parameters of goal-directed motion, as well as playing a role in higher cognitive functions (Kakei et al. 1999). Our findings indicate that the sub-region of the external oblique representation in M1 has greater resting-state connectivity with the superior parietal lobule than the sub-region in SMA. The posterior parietal cortex is increasingly recognized as forming part of the motor system, particularly as the locus of the transformation of sensory input into action-specific information (Fogassi and Luppino 2005). The superior parietal lobule in particular may be associated with visuomotor transformations and localization of the body in space during visually-directed voluntary movements (Fogassi and Luppino 2005). The functional connectivity that we demonstrate between M1 and the primary somatosensory cortex may also be associated with activation of external oblique during goal-directed movement. Previous research has established that direct input from S1 to M1 is important both for executing voluntary motor actions and for learning new motor tasks (Borich et al. 2015). Our findings of functional connectivity between M1 and sensorimotor cortex and posterior parietal cortex support earlier work utilizing a non-specific motor cortex ROI to probe functional connectivity in healthy adults (Park et al., 2011).

All neuroimaging modalities have inherent limitations. TMS provides a direct measurement of corticomotor excitability for the muscle of interest, but the properties of the

motor map are influenced by coil orientation (Laakso et al. 2014), stimulus intensity (Thordstein et al. 2013) current spread, and by the depth of the stimulated neurons within the complex topography of the motor cortex (Thickbroom et al. 1998). Mapping muscle representation with TMS provides a two-dimensional map at the scalp surface that must then be projected onto the cortex if TMS data are to be combined with MRI data. Task-based fMRI is an indirect measure of neuronal activation. Mapping muscle representation with fMRI provides moderate three-dimensional spatial resolution, but also results in diffuse activation in areas such as the sensory cortex that may skew mapping results if ROIs are not carefully determined. Existing research investigating mapping of the hand and foot musculature indicates that motor maps determined utilizing neuro-navigated TMS, and task-based fMRI demonstrate spatial congruence but also highlights that they may in part be quantifying the activation characteristics of different populations of neurons (Herwig et al.2002; Lotze et al. 2003). Therefore, combining these two complementary modalities may overcome the limitations of each individual approach and yield more valid results. Resting-state functional connectivity analyses provide an indirect measure of spontaneous coherent activity in regions with similar functional properties (Fox et al. 2012). While resting-state functional connectivity between two brain regions is in part a function of their structural connectivity, it is also sensitive to indirect connections via other brain areas (Damoiseaux & Greicius, 2009). Resting-state analyses have been demonstrated to predict relevant task-based activation and connectivity (Deluca et al., 2006; Tavor et al., 2016) while avoiding the potential confounds associated with task-based BOLD signal analyses (Fox et al. 2012). However, rs-fMRI analyses are also sensitive to the resting-state conditions under which the data were collected (Buckner et al. 2013), and possibly a selection bias to participants able to best undergo MRI procedures. Additional work is needed to clarify how patterns of functional connectivity during voluntary activation of the trunk musculature may differ from that of the resting-state.

In the present study, TMS and task-based fMRI data were only collected during a voluntary abdominal contraction. Ongoing work in developing MRI-compatible postural challenges for the trunk musculature (Lomond et al. 2013) will allow us to establish how sub-regions within the motor cortical representation of trunk muscles are differentially activated during postural and voluntary motor tasks, and if there are adaptations in functional connectivity during voluntary compared to postural activation. Although we selected to quantify representation of external oblique in this study, we do not anticipate that the results would be significantly different for other muscles of the trunk that have similar cortical representations and function (O'Connell et al. 2007; Tsao et al. 2011). However, additional research is needed to determine how the representation in SMA and M1, and associated functional connectivity, may differ in appendicular musculature that does not have a significant postural role.

The ROI identified in this paper could be used to test the association between resting-state functional connectivity and active muscle control in future studies in healthy individuals and individuals with musculoskeletal or neurological dysfunction. Resting-state functional connectivity could be measured in individual participants between our motor cortical ROI and discovered ROI in basal ganglia, cerebellum, sensory and parietal cortex. We would predict inter-individual differences in these resting-state functional connectivity measurements would be associated with inter-individual differences in task performance. For example, we would predict that participants with stronger SMA-basal ganglia functional connectivity may have enhanced performance on tasks involving anticipatory postural adjustments (Ng et al. 2011). Individuals with chronic low back pain have altered postural activation of the trunk musculature, including external oblique (Silfies et al. 2009), and altered resting-state functional connectivity in regions associated with pain perception, in the default mode network and in sensorimotor regions including SMA, M1 and lobules IV and V of the cerebellum (Baliki et al.2013; Baliki et al. 2008; Pijnenburg et al. 2015). Therefore, future work may also probe if chronic low back pain is

associated with altered resting-state SMA-basal ganglia/cerebellar functional connectivity and if this correlates with impaired anticipatory postural adjustments in these individuals.

Our current study represents an important first step towards determining how activity of different regions of motor cortex may coordinate with distributed brain areas to produce functionally adapted activity in a muscle under varying task contexts.

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CAPTIONS TO FIGURES

Fig 1 Motor cortical representation and construction of regions-of-interest (ROI). (a) Heat map shows averaged MEP magnitude across all participants, with eight circled locations showing where MEP magnitude was significantly greater than other locations. We show example MEP traces from an individual participant at three of these stimulation points (red, green, and blue) along an anterior-posterior axis. (b) Stimulation locations overlaid on an fMRI activation map from voluntary contraction of EO. Regions nearest to the exemplar red, green, and blue stimulation points differed in the composition of Brodmann areas (BA) at the voxel level; the red stimulation point was closest to voxels associated with BA 6, the green stimulation point was closest to voxels associated with a mixture of BA 4 and 6, and the blue stimulation point was associated with primarily BA 4 voxels. None of the red, green, or blue stimulation points had a contribution from voxels primarily associated with sensory cortex (BA 1-3). (c) We constructed ROI for resting-state functional connectivity analysis by finding all voxels closest to the eight stimulation points with significant MEP, that activated during voluntary EO contraction, and were more associated with BA 6 (red ROI) and BA 4 (blue ROI)

Fig 2 Distinct functional connectivity of regions-of-interest (ROI) in the motor cortical representation of the external oblique (EO) muscle. (a) We found regions in the discovery group for which functional connectivity with the EO representation in BA 6 was greater than the functional connectivity with the EO representation in BA 4. For these regions, we highlight the basal ganglia (putamen) and cerebellum for further analysis. We also found regions in the discovery group (100 participants) for which functional connectivity with the EO representation in BA 4 was greater than the functional connectivity with the EO representation in BA 6. For these regions, we highlight somatosensory cortex and parietal cortex for further analysis. Heat

maps show effect size (Cohen's d) for BA6>BA4 and BA4>BA6. z value is the mm coordinate of the axial slice in standard MNI coordinates. (b) Validation of the functional connectivity findings on the discovery group on an independent sample of 100 participants (validation group). We extracted an average functional connectivity value in the clusters defined in (a) (putamen, cerebellum, somatosensory, and parietal), we show the distribution in the discovery group for reference, and then show the distribution in the validation group to confirm the reproducibility of the results ($p<0.0001$ ****). Box plot whiskers show 99th percentile, and (+) show outliers