

Structural brain changes after 4 wk of unilateral strength training of the lower limb

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¹MI-Lab, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway; ²Department of Medical Imaging, St. Olavs Hospital, Trondheim, Norway; ³Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway; ⁴Department of Physical Medicine and Rehabilitation, St. Olavs Hospital, Trondheim, Norway; ⁵Hokksund Medical Rehabilitation Centre, Hokksund, Norway; and ⁶Department of Sports and Outdoor Life Studies, Telemark University College, Bø, Norway

Submitted 2 March 2012; accepted in final form 12 March 2013

Palmer HS, Håberg AK, Fimland MS, Solstad GM, Iversen VM, Hoff J, Helgerud J, Eikenes L. Structural brain changes after 4 wk of unilateral strength training of the lower limb. *J Appl Physiol* 115: 167–175, 2013. First published March 14, 2013; doi:10.1152/jappphysiol.00277.2012.—Strength training enhances muscular strength and neural drive, but the underlying neuronal mechanisms remain unclear. This study used magnetic resonance imaging (MRI) to identify possible changes in corticospinal tract (CST) microstructure, cortical activation, and subcortical structure volumes following unilateral strength training of the plantar flexors. Mechanisms underlying cross-education of strength in the untrained leg were also investigated. Young, healthy adult volunteers were assigned to training ($n = 12$) or control ($n = 9$) groups. The 4 wk of training consisted of 16 sessions of 36 unilateral isometric plantar flexions. Maximum voluntary isometric contraction torque was tested pre- and posttraining. MRI investigation included a T1-weighted scan, diffusion tensor imaging and functional MRI. Probabilistic fiber tracking of the CST was performed on the diffusion tensor imaging images using a two-regions-of-interest approach. Fractional anisotropy and mean diffusivity were calculated for the left and right CST in each individual before and after training. Standard functional MRI analyses and volumetric analyses of subcortical structures were also performed. Maximum voluntary isometric contraction significantly increased in both the trained and untrained legs of the training group, but not the control group. A significant decrease in mean diffusivity was found in the left CST following strength training of the right leg. No significant changes were detected in the right CST. No significant changes in cortical activation were observed following training. A significant reduction in left putamen volume was found after training. This study provides the first evidence for strength training-related changes in white matter and putamen in the healthy adult brain.

neural adaptations; cross-education; magnetic resonance imaging; diffusion tensor imaging

IT IS WIDELY ACCEPTED THAT strength training not only elicits adaptations in skeletal muscle tissue, but also results in neural adaptations. Strength training increases the torque of maximum voluntary isometric contraction (MVC) in the trained limb, as well as the contralateral untrained limb (1, 21, 36, 41, 45). Significant improvements in strength, in the absence of muscular hypertrophy, are elicited following strength training protocols lasting 4–8 wk (23). Strength enhancement in the

untrained limb is termed cross-education and occurs in the absence of muscle hypertrophy. Thus local changes in muscle tissue are insufficient to explain increases in strength, and neural adaptations are thought to have a key role in the effect of strength training of both the trained and cross-educated, untrained limb.

However, the mechanisms underlying neural adaptations to strength training remain unclear, partly due to varying methodologies with regard to both training protocols and measurements used (39). Accumulating evidence supports a role for increase in neural drive to the α -neurons caused by increased corticospinal tract (CST) excitability (28, 29, 53), possibly connected to adaptive changes in primary motor cortex (M1), and/or unmasking of dormant corticospinal connections (2, 8, 11, 35, 36, 56). The idea of central neuronal adaptive processes initiated by strength training is in line with the concept of experience-induced neuroplasticity (57). For example, repeated neuronal electrical activity induces myelination in the central nervous system, leading to experience-driven white matter plasticity; this has been shown in cultured astrocytes over a time course of days to weeks (30). Further in vitro evidence clearly linking neuronal electrical activity to myelinogenesis is the inhibition or enhancement of myelination by manipulating neuronal firing in cultured embryonic mouse brain hemispheres (13). It is speculated that similar processes may occur in humans (59). It is plausible that similar structural changes may have a role in neuronal adaptations to strength training, leading to increased CST myelination.

Adaptations in the M1 are involved in motor learning (34, 51, 60). There is also some evidence that cortical changes have a similar role in the early neural responses to strength training (8, 56), probably mediated by long-term potentiation. It appears that adaptive changes in M1 occur rapidly and may occur even in response to only one session of strength training (56). Strength training-induced changes in cortical activation have been demonstrated previously using blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) in a study of arm strength (17). Farthing and colleagues (17) provided some evidence for strength training-induced changes in BOLD fMRI activation area in the M1 following training, but they did not use standard fMRI analysis techniques and only tested four individuals.

Alterations in subcortical structures are also considered to be involved in neuronal adaptations (56). There is no direct evidence for subcortical gray matter nuclei being involved in increased strength, but professional ballet dancers have signif-

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icant differences in gray and white matter volumes compared with controls (25). Hänggi and colleagues (25) reported decreased gray matter volumes in several structures of the sensorimotor network in dancers compared with nondancers, including the putamen, a subcortical structure within the basal ganglia, which is thought to be involved with movement (3, 12, 43) and learning (48). This, along with other similar and related findings in both humans (37) and rats (44), suggests that experience-driven changes may also occur in subcortical structures, such as the basal ganglia.

In short, the supraspinal mechanisms behind the strength training-induced increase in neural drive to trained and untrained muscles remain to be determined. The present study was based on the hypothesis that changes in white matter microstructure would occur in CST following strength training due to repeated stimulation of the same pathway. Experience-driven changes in white matter microstructure can be measured with diffusion tensor imaging (DTI) (55).

In light of the findings of Farthing and colleagues (17), it was also hypothesized that strength training would result in increased fMRI activity in M1. The present study also aimed to explore changes in gray matter subcortical nuclei and hypothesized that, in particular, a volume change in putamen, which is involved in movement regulation and learning, would occur following strength training. Strength training-induced changes have, to date, mainly been investigated in the contralateral and not the ipsilateral hemisphere. The present study investigates central nervous system changes in both the contralateral and ipsilateral hemisphere in an attempt to find mechanisms to explain the cross-education effect.

MATERIALS AND METHODS

Subjects. Twenty-one healthy, recreationally active volunteers (24 ± 2 yr old; mean \pm SD) with no history of neurological disorders, head trauma, current Diagnostic and Statistical Manual of Mental Disorders-IV axis I diagnosis of psychiatric illness, or substance abuse were recruited from the university campus. Participants filled in a physical activity questionnaire to determine that they were all recreationally active to a similar degree and did not do any strength training of their legs before or during the study. The level of self-reported weekly recreational physical activity of the participants, including estimated weekly walking, was 7.9 ± 2.1 h (mean \pm SD, $n = 21$), there was no difference in the baseline self-reported physical activity level between the training (8.2 ± 2.4 h) and control (7.5 ± 1.8 h) groups ($P = 0.3$, Student's *t*-test; unless otherwise stated all statistical tests used a *P* value threshold of 0.05 to determine statistical significance). All participants were instructed to continue with their normal recreational physical activity and not begin any other strength training exercise until after the study was completed. The volunteers were randomly assigned to a training group ($n = 12$; 8 women, 4 men) or to a control group ($n = 9$; 5 women, 4 men). This MRI study was a substudy of a previously published study (21) of EMG and reflex excitability responses to unilateral strength training.

Handedness was assessed using the Edinburgh Handedness Inventory (47). Foot preference (10, 24) was determined using the Waterloo Footedness questionnaire, revised (16). The training group and control group underwent anatomical (T1-weighted), functional (T2* BOLD fMRI), and DTI scans before commencing and after completing the strength training intervention, as detailed below.

The study was approved by the Regional Committee for Medical Research Ethics at the Norwegian University for Science and Technology, Trondheim, Norway. All subjects gave their written, informed consent for all parts of the study.

Strength training. The training intervention consisted of 16 sessions of plantar flexion MVC training over a 4-wk period. Training group subjects performed unilateral strength training of their dominant leg, as detailed below. For all but two of the subjects, the dominant leg was the right leg. For the two left-footed subjects, all of the brain images were flipped before further analyses. Throughout the rest of this article, the dominant leg can be assumed to be and is referred to as the right leg to avoid confusion. The apparatus used and training conducted were as previously described (21). Briefly, the subjects were placed in a slightly reclined chair mounted on a solid wooden platform with one foot placed in a custom-made ankle dynamometer. Rigid straps secured the heel and forefoot to the footplate with the ankle at 90° . The opposite limb was resting on a chair. One of the test leaders ensured that the subject did not perform unwanted muscle activity. In each training session, training group subjects performed warm-up repetitions and six sets of six MVCs. Each MVC lasted 4 s and was separated by a 10-s rest interval. During MVC repetitions, subjects could visually observe the contraction force on a computer screen and received enthusiastic verbal encouragement. Before and following the training intervention, MVC force was measured from both legs and recorded by a force transducer (model 363-D3-50-20P1, Revere Transducers, Tustin, CA), as previously described (21). This study used a passive control group who did not undergo any intervention; the volunteers merely underwent all pre- and posttests following the same procedures as the training group. The control group volunteers were all right footed.

Data analysis: strength. MVC torque was calculated as the average of the two best attempts (highest peak force) from seven MVCs recorded pre- and posttraining. These data were normalized to percent change from pre- to posttraining. Two-way ANOVA (SigmaPlot, Systat Software, Erkrath, Germany) was used to test for significant differences in mean percent change between the groups; that is, to test for main effects of the two factors: group (training and control) and limb (dominant and nondominant). In the case of significant effects, pairwise multiple comparison procedures were performed using the Holm-Sidak method to test which groups differed significantly from each other.

DTI acquisition. The DTI sequence was a single-shot balanced echo planar imaging sequence acquired in 12 non-collinear directions with $b = 1,000$ s/mm² using the following parameters: repetition time = 10 400 ms, echo time = 100 ms, field of view = 260×260 mm, slice thickness = 2.5 mm, acquisition matrix = 102×102 , giving isotropic voxels of 2.5 mm. Forty transversal slices with no gap were acquired, giving full brain coverage. For each slice, 1 image without diffusion weighting ($b = 0$), and 12 images with diffusion weighting were acquired. The DTI sequence was repeated four times for increased signal-to-noise ratio. Due to technical problems and time constraints that occurred during the posttesting, posttraining DTI images were not acquired for two of the control group subjects who participated in the MRI study. In addition, the DTI images for the two left-footed volunteers were excluded from the DTI analyses, rather than flipping, the images as was done for the fMRI data. Therefore, the group sizes remaining for the DTI analyses were $n = 10$ for the training group and $n = 7$ for the control group.

Data analysis: DTI. DTI analysis was performed with the tools of the FMRIB software library (FSL, Oxford Centre for Functional MRI of the Brain, UK; www.fmrib.ox.ac.uk/fsl). Image artifacts due to motion and eddy current distortions were minimized by registration of all the DTI acquisitions to the $b = 0$ image using affine registration. The brain was extracted using Brain Extraction Tool (part of FSL). FMRIB's diffusion toolbox was used to fit a diffusion tensor model to the raw diffusion data. Voxelwise maps of fractional anisotropy (FA) and mean diffusivity (MD) were calculated for all of the four DTI acquisitions for the trained group and control group and finally averaged to get the mean of the diffusion metrics from the four acquisitions. FA describes the degree of directional dependence of a diffusion process. In DTI, the FA value is assumed to reflect fiber

density, axonal diameter, and myelination in white matter. MD is a scalar measure of the total diffusion within voxels. In DTI tractography, FA and MD values reflect the strength of white matter tracts.

Probabilistic tracking. Fiber tracking of the CST to M1 foot area was performed using a probabilistic tractography routine implemented in FSL based on a multifiber model (5,000 streamline samples, 0.5-mm step lengths, curvature thresholds = 0.2) (5). A two-regions-of-interest (ROI) approach was used to select the CST fibers connecting to the M1 foot area, as determined from the fMRI data in the left and right hemispheres separately. The first seed ROI was placed in three contiguous slices at the cerebral peduncle, corticospinal tract (46), on each individual's color-coded FA map. To ensure that the ROI was placed in the same anatomical region in the pre- and posttraining FA image for the same individual, the pre- and posttraining ROI masks were registered to each other and added together in each individual's diffusion space. The second ROI was placed in the foot motor area in the cortex of each individual's FA map. The primary motor area in the brain activated during plantar flexor contractions in all subjects was identified from the fMRI data by defining group activation maps using FSL's fixed effects analysis and using the resulting activated area as the ROI for M1. Primary motor activation areas were defined in this way in the left and right hemisphere, derived from the fMRI data taken during plantar flexor contractions of the dominant right leg and nondominant left leg, respectively (Fig. 1). These motor activation areas defined by the fMRI data were linearly transformed from Montreal Neurological Institute (MNI) space to FA (diffusion) space using FSL's FLIRT (FMRIB's Linear Image Registration Tool) (31, 32) (Fig. 2). Only the streamlines that passed through the two ROIs were included in the analysis. An exclusion mask was also included in the midline of the brain dividing the two hemispheres, and pathways were discarded if they entered the exclusion mask. The resulting fiber structures were visually inspected for each individual (Fig. 3). The mean FA and MD were calculated for the left and right CST in all control and trained individuals before and after training. MD and FA values were normalized to percent change from pre- to posttraining scans. *T*-tests (parametric) or Mann-Whitney rank sum tests (nonparametric) were used to compare the percent change in MD and FA values between the training and control groups (SigmaPlot). In addition, regression analyses were performed to determine whether correlations existed between percent change in MD or FA values in the CST and percent change in MVC in the contralateral leg following training.

fMRI paradigm and acquisition. The paradigm was compiled in, and actual visual presentation times logged by, E-prime (Psychology Software Tools). MRI data were acquired on a 3 T Siemens Trio with Quantum gradients (30 mT/m) and a 12-channel phased array vendor-supplied head coil (Siemens, Erlangen, Germany). Foam pads were used to minimize head motion. The fMRI stimuli were presented using MRI-compatible LCD goggles (Nordic NeuroLab, Bergen, Norway). T2*-weighted BOLD images were acquired using an echo-planar imaging pulse sequence (repetition time = 2,700 ms, echo time = 35 ms, field of view = 244 mm × 244 mm, slice thickness = 3.0 mm, slice number = 45, matrix = 80 × 80, giving an in-plane resolution of 3.05 mm × 3.05 mm). Each functional run contained 141 volumes acquired in the transverse plane. Image acquisition and stimulus presentation were synchronized with a sync-box (Nordic Neuro Laboratories, Bergen, Norway). T1-weighted image consisting of 160 contiguous slices of 1.2-mm thickness with an in-plane resolution of 1.0 × 1.0 mm² was acquired for anatomical reference and for segmentation for volumetric analysis of subcortical structures.

The fMRI stimuli presented visually consisted of a black footprint symbol and a blue rectangle. The black footprint symbol indicated isometric plantar flexion contraction. Contractions were similar to those performed during the pre- and posttests of MVC and during training (training group only), but lying supine rather than seated. Subjects were instructed to perform as powerful a contraction as possible without moving their head. To help prevent head movement

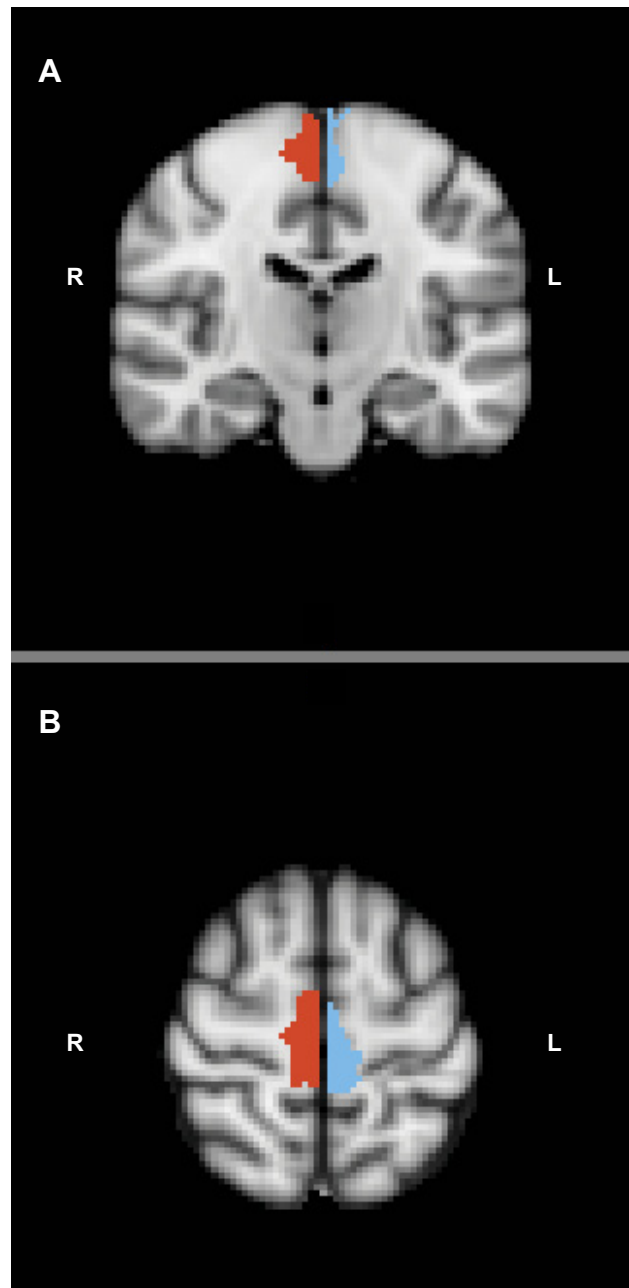


Fig. 1. Primary motor cortex activation areas identified from the functional magnetic resonance imaging (fMRI) analysis displayed on Montreal Neurological Institute (MNI) standard space in coronal (A) and axial (B) planes. Red area, activation in right (R) side of brain during plantar flexion of the left leg/foot; blue area, activation in left (L) side of brain during plantar flexion of the right leg/foot. These activation areas were used as the second region of interest (ROI) for the probabilistic tracking of the corticospinal tract (CST).

during scanning, padding was used around the head and strapping was used around the subjects' bodies. Since the strapping used to secure the foot, to facilitate an isometric contraction, was rather tight, volunteers during pilot testing reported that their strapped foot became uncomfortable during scanning. Therefore, in the present study, subjects wore correctly fitting snowboard boots during scanning to increase comfort and task compliance. Participants received instruction before both pre- and posttraining scanning and repeated the contraction under instructed supervision until complying with desired task instruction, with particular emphasis on performing the contractions without moving the rest of the body.

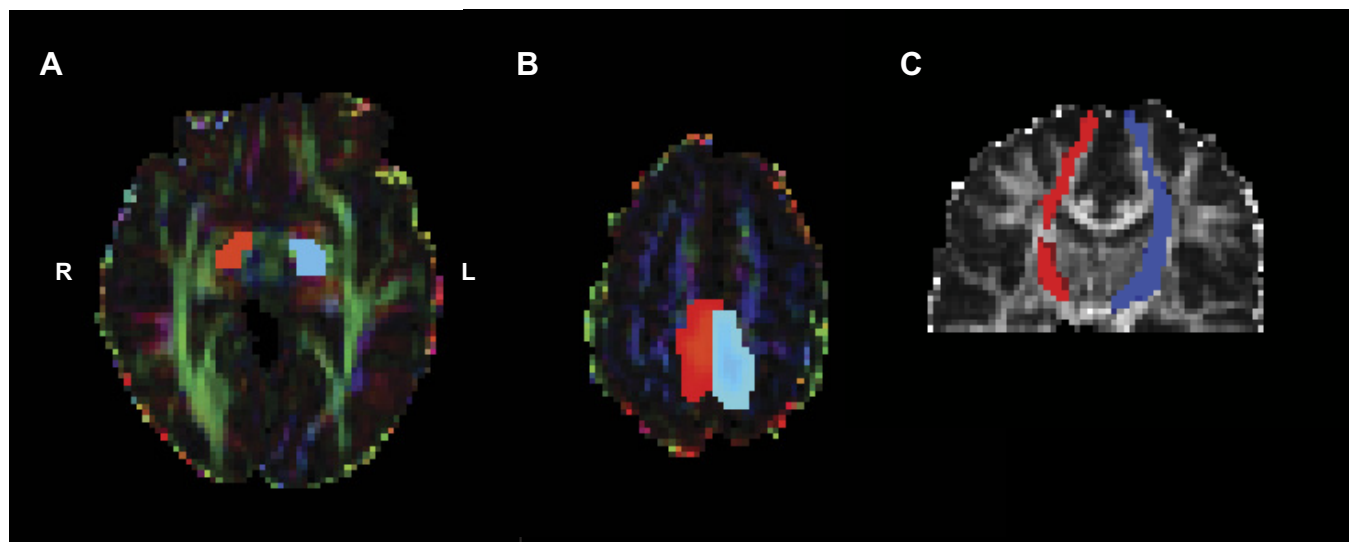


Fig. 2. Seed ROIs in the L (blue) and R (red) hemisphere placed at the cerebral peduncle (A) and blood oxygenation level-dependent fMRI activation areas (B) linearly transformed from MNI space to diffusion space and overlaid onto an axial color-coded fractional anisotropy (FA) map of one representative individual. C: probabilistic fiber tracking results, from the two ROIs shown in A and B, of the CST overlaid onto a coronal FA map of one representative individual.

The force of the contractions was not measured, since MRI-compatible equipment for measuring this was not available. The presentation of the blue rectangle indicated that subjects should release the contraction and rest. The footprint symbol was presented

for 14 s, and the rest symbol was presented for 26 s. Subjects performed six contractions preceded by rest periods. Subjects performed two separate fMRI paradigms: one with isometric plantar flexion contractions of the dominant right leg, and another with

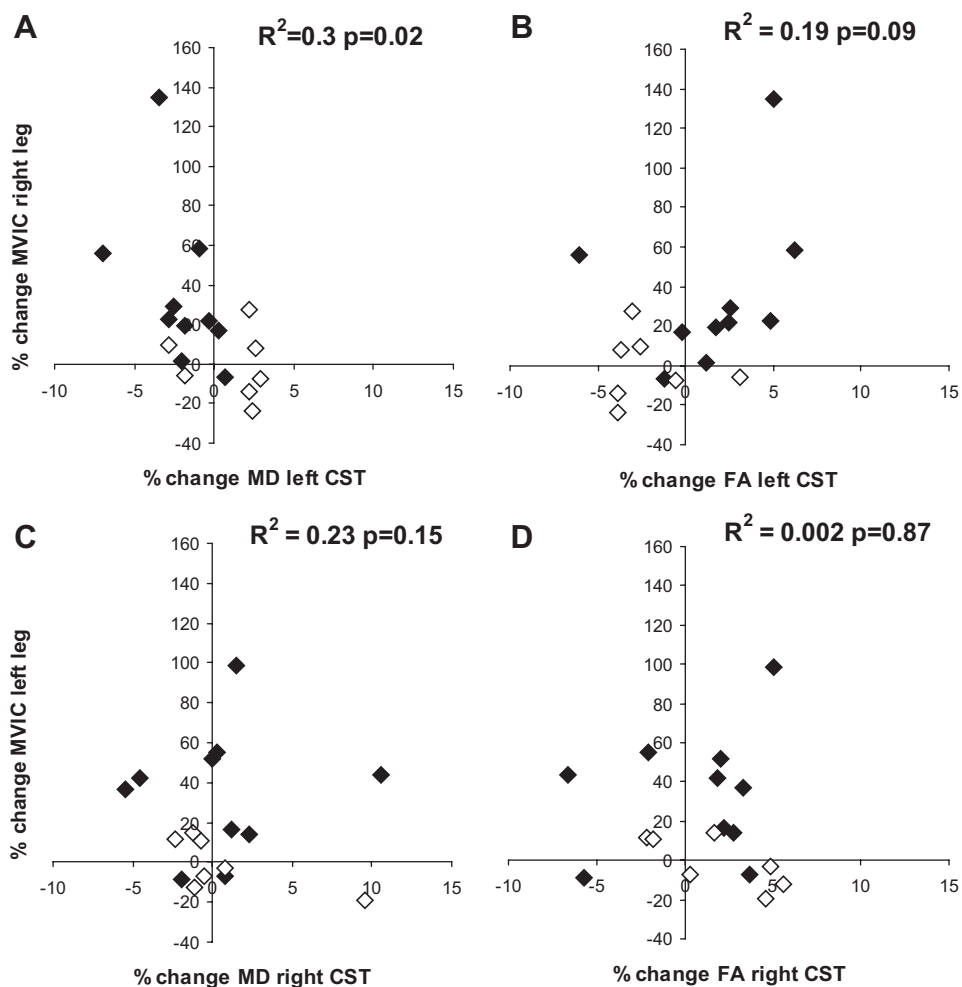


Fig. 3. Regression plots of percent change in mean diffusivity (MD; A and C) and FA (B and D) and percent change in maximum voluntary isometric contraction (MVIC) in the right (A and B) and left (C and D) leg in training (◆) and control (◇) subjects.

plantar flexion contractions of the nondominant left leg. The fMRI experiment was carried out before and after the training intervention within 3–5 days of subjects completing the required 16 strength training sessions.

Data analysis: fMRI. All fMRI analyses were performed in FSL 4.0 (42) (FMRIB Software Library, Oxford, UK; www.fmrib.ox.ac.uk/fsl/). The functional images were motion corrected, spatially smoothed (Gaussian kernel full width at half-maximum; 5 mm), and high-pass filtered (cutoff 100 s). The functional images were registered to the high-resolution anatomical T1W three-dimensional volume and, before group analysis, warped to the MNI-152 standard template. The registration was performed with FLIRT (31). Time course statistical analyses were performed using the general linear model. The expected signal time courses corresponding to the given visual instruction stimuli were modeled with a box-car stimulus response function, convolved with a double-gamma hemodynamic response function and its temporal derivative. Two approaches were used to statistically analyze the fMRI data set. Traditional whole-brain group analysis was conducted as follows: group statistics were calculated with a mixed-effects model of variance, as implemented in FLAME1 (FMRIBs local analysis of mixed effects) (4). The planned contrasts were tested for significance with a cluster threshold of $z > 2.3$ and $P < 0.05$ (corrected for multiple comparisons).

In addition, the data were analyzed based on the activation within an anatomical ROI in the M1 for the foot/shin. Anatomical masks of the paracentral lobes were created based on the atlas of anatomical labels (58) in the Wake Forest University Pick Atlas (<http://www.ansir.wfubmc.edu/>) (42) in SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>). Using these ROIs, parameter estimates (beta values) of activation within the masks were determined using FSL's featquery tool, separately for the left and right side of the brain. Groupwise statistical analysis was performed on the resulting parameter estimates (beta values). The parameter estimates (beta values) within the ROI were normalized to percent change in activation before and after training and statistical analyses were also performed on these. Regression analyses were performed to investigate potential correlations between percent change in parameter estimates of activation and percent change in MVC in the contralateral leg following training for both the left and right side of the brain and corresponding contralateral leg.

Volumetric analysis of subcortical structures. Volumetric analysis of subcortical structures was performed on the T1-weighted three-dimensional images of all subjects, before and after training, using FSL's FIRST (52). The number of voxels and volume in cubic millimeters of all subcortical structures was derived. The full list of segmented structures that were analyzed volumetrically in the present study is as follows: brain stem/fourth ventricle and both left and right thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and accumbens. Paired *t*-tests were used to test for significant differences in the number of voxels and volume in cubic millimeters of the segmented subcortical brain structures before and after the strength training protocol. In the event of a significant change in subcortical structure volume following training, regression analysis was performed to determine whether there was any correlation between percent change in volume and percent change in MVC in the contralateral trained or untrained leg. All statistical tests used a *P* value threshold of 0.05 to determine statistical significance.

RESULTS

Strength. Strength training increased MVC in the training group's trained and untrained leg. The percent increase in MVC, between the pre- and posttraining tests, by the training group was $39.5 \pm 39.7\%$ (mean \pm SD) for the dominant, trained leg and $30.1 \pm 30.8\%$ for the nondominant, untrained leg. Two-way ANOVA of the percent change in MVC data

showed a statistically significant difference ($P < 0.001$) between the percent change in MVC of the training and control group and no significant difference in the mean percent change MVC values for the dominant and nondominant limb ($P = 0.3$). Pairwise multiple comparison procedures (Holm-Sidak method) showed the percent change in MVC of the training group differed significantly from the control group for both the dominant and the nondominant limb (both $P < 0.001$).

DTI. The training group showed a significant decrease in MD of $2.0 \pm 2.3\%$ in the left CST (mean \pm SD; $P = 0.021$, *t*-test, Cohen's $d = 1.38$) following the strength training intervention. There were no significant changes in MD in the training group's right CST or in either CST of the control group. There was a significant difference in the percent change in MD between the training and the control group ($P = 0.014$, *t*-test). The percent decrease in MD in the left CST of all of the training group subjects was significantly correlated ($P = 0.02$, $R^2 = 0.3$) to the percent increase in MVC in the trained (right) leg (Fig. 3A).

The training group demonstrated a $1.7 \pm 3.6\%$ not significant increase in FA in the left CST after strength training of the right leg (mean \pm SD; $P = 0.7$, *t*-test, Cohen's $d = 1.2$). The increase in FA in the left CST of the training group differed significantly from the percent change in the left CST of the control group ($P = 0.01$, *t*-test). There was no significant correlation between percent change in FA in the left CST and percent change in MVC in the trained right leg ($P = 0.09$ and $R^2 = 0.19$; Fig. 3B).

fMRI activation. Neither of the two approaches used in the present study found any significant changes in BOLD fMRI activation following the strength training intervention. Visual inspection of the group activation maps suggested that there may be some differences in cortical activation during isometric plantar flexion contraction following strength training, for example, more activation in the premotor cortex following strength training. However, when statistically contrasted using standard fMRI analysis methods, there was no significant difference in BOLD activation pre- and posttraining. ROI analysis using featquery (FSL) of the fixed effects activation revealed no significant group changes in activation in the M1 (foot/lower leg) ROI following training. Regression analyses revealed no significant correlations between activation in the ROI pre- and posttraining and change in MVC in the contralateral legs.

Volumetric analysis of subcortical brain structures. In the training group, a significant reduction in the volume of the left putamen was found after training ($2.0 \pm 2.5\%$ decrease, mean \pm SD; $n = 12$, $P = 0.016$, Cohen's $d = 0.73$). No significant changes were found in the right putamen or in any of the other subcortical brain structures in the training group. No significant volume changes were found in the control group in any of the subcortical structures. There was no significant correlation between percent change in volume of the left putamen and percent change in MVC in the right leg after strength training.

DISCUSSION

The main findings of the present study are that unilateral strength training of the plantar flexors in healthy, young adults induced contralateral changes in the CST foot fibers and decreased putamen volumes. No changes in brain structures

were observed in the hemisphere controlling the untrained leg. This suggests that 16 sessions of 36 voluntary repetitions is an adequate stimulus to induce contralateral brain changes in both white matter and subcortical gray matter in the hemisphere controlling the trained leg. Despite a significant increase in strength in the untrained leg, structural brain changes were not observed in the hemisphere controlling the untrained leg. When one thinks about experience-driven neural plasticity in the brain, it is usually more complex motor or cognitive tasks that are considered important.

The present study demonstrates that even a relatively simple motor task, when performed repeatedly with contractions approaching maximal voluntary force, induces small (~2%) but significant changes in both white matter and subcortical gray matter. It remains to be determined whether repeated contractions performed with weaker forces would have elicited similar changes observed with neuroimaging. The possible contribution of motor learning to these results is discussed further below. However, physiological interpretation of changes in measures derived from neuroimaging is challenging and must be treated cautiously as a number of biological processes may be involved. Changes observed by neuroimaging may be accompanied by changes in neuronal discharge within and between brain structures involved in movement, but further research using multiple techniques will be needed to elucidate the mechanisms underlying these adaptations.

The present study found that MD in the left CST was significantly reduced after strength training of the right plantar flexors. In addition, a trend for increased FA in the left CST after strength training of the right leg was found. Localized increases in FA have previously been shown following juggling training, a complex visuo-motor task (55), but this study presents the first evidence for white matter changes following training of a single movement. A significant reduction in MD and an increase in FA may indicate increased coherence of the CST fibers that were included in this analysis, increased myelination, or increased axon density (9, 50, 54). Based on the relatively short duration of the training intervention in this study (4 wk) and previous evidence showing that myelination (13, 30) can change in a relatively short time period, it is suggested that this factor may be the most likely to have played a role in the observed changes in DTI measures. Axonal reorganization, in particular, is less likely to be involved, as it is thought to occur over a longer time period and has more often been associated with developmental adaptations (40). Repeated neuronal electrical activity induces myelination in the central nervous system; this has been shown in cultured astrocytes (30). Further *in vitro* evidence clearly linking neuronal electrical activity to myelinogenesis is the inhibition or enhancement of myelination by manipulating neuronal firing in cultured embryonic mouse brain hemispheres (13). It is speculated that similar processes may occur in humans (59). Electrical activity in an axon has been shown to regulate its myelination over a time course of days to weeks (13, 30). Myelin regulates conduction velocity and synchronization of neuronal firing (18). An increase in neural drive to the plantar flexor muscles has been shown by our colleagues as increased EMG activity and increased V-wave amplitude in the soleus muscle of the trained and untrained leg (19). Taken together with the above, it seems plausible that increased myelination of these CST fibers may underlie an increased transmission rate of

activation to the trained leg. Changes in MD and FA in the CST may, therefore, contribute to neuronal adaptation to strength training by altering conduction velocity and synchronization of firing.

It is pertinent to highlight that, because of the two-ROI approach that was used in this study, with the cortical ROI derived from the group fMRI data taken at the same time points, the fibers included in the tractography are limited to those likely to be involved in the neuronal firing that results in plantar flexion contraction, rather than the entire CST. In the context of the present study, this methodology is likely to be a more powerful way of detecting microstructural changes in white matter, compared with investigating the entire CST and more specific to this experimental question.

The change in white matter microstructure seen in this study, a reduction in MD and an increase in FA, appears to be somewhat correlated to the magnitude of the strength training response, as measured by increased MVC by the plantar flexor muscles. In training studies, it is important to look for correlations such as these rather than to simply consider the group average, since individual variation in the response to the training stimulus can be large (6, 26, 33). This weak but significant correlation indicates that, at least to some extent, the individuals who responded the most to the strength training stimulus, with larger increases in MVC, also showed greater changes in white matter microstructure in the CST.

However, despite some correlation between the magnitude of the strength training response and the changes detected with imaging, the contribution of learning (49), rather than strength training adaptations, cannot be ruled out without conducting a further study. Such a study should include a motor learning group who learned the motor task by performing it at a reduced effort, for example 20% of maximal effort. Motor learning is likely to have a key role in the changes observed in the present study.

An interesting dichotomy is presented since this study found no evidence for cross-education resulting in significant changes in brain structure or activity in the ipsilateral hemisphere, although a marked increase in strength was demonstrated in the untrained leg. The impressive magnitude of the cross-education effect shown in the present study, and reported previously by our colleagues (21), in terms of the strength enhancement in the untrained leg (30%) is underlined by the fact that there was no significant difference in the percent increase in MVC between the trained and untrained leg. This degree of cross-education is larger than shown in other studies (7). This difference may be explained by the fact that the present study used a high training load, with each repetition being close to the volunteer's MVC. In addition, the majority of cross-education studies have been done in the upper limbs, and it is not uncommon to find a larger strength training response and cross-education effect in the lower limb (39). The mechanism underlying the cross-education effect remains unclear. Despite a significant increase in strength in the untrained leg, structural brain changes were not observed in the hemisphere controlling the untrained leg. It may be possible that spinal cord neurons controlling the untrained leg somehow become excited by the CST of the trained leg. Faster transmission along descending motor pathways to the trained leg could potentially interact with the untrained leg at the spinal level.

For this strength training paradigm, no significant changes in cortical activation occur at the whole brain level or in the ROI analyses in the M1. This could have been due to the type of contraction performed by the volunteers during fMRI. A previous study (17) did report changes in cortical activation following strength training of the ulnar deviator muscles. However, Farthing and colleagues (17) used a small group of volunteers ($n = 4$) and very different methodology. It is possible that changes in M1 activity occur earlier in the adaptation to strength training (56) and are, therefore, no longer detectable after 4-wk strength training. In addition, there are fewer cortical inputs to distal muscle groups that move the foot/leg than for the hand/arm muscles. There may also be lower reproducibility of foot than hand fMRI, although one study has shown the reproducibility of fMRI activation to foot and hand motor paradigms to be similar (27).

Another factor potentially affecting the fMRI study is that the six repeated strong plantar flexion contractions performed during fMRI scanning may have induced fatigue. Although volunteers did not report that the series of contractions was fatiguing, force output from the muscle was not measured during the fMRI experiment. The force of contractions across the six stimulus blocks may have reduced if the volunteers became fatigued. If fatigue did occur during the fMRI acquisition, changes in cortical activation may have occurred between the six stimulation blocks. It is difficult to predict how such an effect would have affected the pre- and posttraining group comparison, but, if fatigue differentially affected the volunteers, this may have given rise to more heterogeneity in the group analyses. A positron emission tomography study of biceps brachii MVCs suggests that cortical activity may increase in the case of fatigue (38). Thus, if the training group were more resistant to fatigue after training, one might expect a reduction in cortical activation. However, another positron emission tomography study, which used finger movements as the motor task, suggests that application of greater forces is associated with increases in regional cerebral blood flow in the M1 (14). The training group may have performed the isometric plantar flexion movements with greater force after training; these effects could be investigated further, if force was measured during fMRI acquisition.

In addition to white matter changes in the left CST, a significant reduction in the volume of the left putamen was found in the training group after strength training. The putamen is a subcortical structure within the basal ganglia, which is thought to be involved with movement (3, 12, 43) and learning (48). The reduction in left putamen volume in the present study corresponds to a previous study of trained ballet dancers who were found to have decreased gray matter volume in several structures of the sensorimotor network, including the putamen, compared with nondancers, even when these data were corrected for the dancer's lower body weight (25). In addition, forced right-handedness during childhood has been associated with reduced volume of the left putamen in adult "converted" left-handers (37). Both of these studies implicate reduction in putamen volume in the adaptation to environmental challenges, possibly related to pruning processes during motor development. However, the present study is, to our knowledge, the first to show changes in putamen volume in response to a repeated motor challenge over time frame of just 4 wk. A related finding is the reported increase in optical density of the dorsolateral

caudate putamen in rats after 6 mo of voluntary exercise (44). Taken together, these findings support caudate plasticity in response to changes in motor behavior. The present study adds to existing knowledge of this phenomenon by suggesting that volume changes to human adult putamen can occur in just a few weeks, given a sufficient motor challenge. The present view is that the underlying mechanisms for the structural plasticity, observed with neuroimaging techniques as volume changes, involve capping glia, dendrite remodeling, changes in dendritic spine density, and associated synaptic changes (15). Since the change in left putamen volume did not correlate to training outcome, one could speculate that the changes in putamen are related to the motor-learning component of the adaptation, whereas the significant DTI outcome, which did correlate to strength enhancement, is associated with strength. Further studies investigating the motor learning and strength components separately are required to test such a hypothesis.

Knowledge concerning neural adaptations to strength training is of clinical importance, especially for patient groups with neurological injury or disease. In stroke patients, it was recently reported that muscle activation capacity of the paretic plantar flexors was only one-third of the nonparetic limb (22). Large improvements in strength have been reported after strength training for this population, to a large extent due to neural adaptations. In multiple sclerosis patients, a recent study reported augmentation of efferent motor drive from spinal motor neurons to plantar flexors (20). However, the mechanisms underlying strength improvements and enhanced motor drive must be better understood to prescribe effective rehabilitation regimes for neurological patients. Studies of strength training of both the upper and lower limb should be undertaken, since the mechanisms may differ, and deficits in upper and lower limb function differentially affect quality of life.

In conclusion, the present study has shown that unilateral strength training of the plantar flexors over a relatively short time frame (16 sessions in 4 wk) with a relatively limited time commitment (each session had a total time commitment of ~15 min) is adequate to elicit improvements in strength in both the trained and untrained leg and changes in both contralateral white and gray matter in the brain. Most notably, unilateral strength training elicited significant microstructural changes in the contralateral CST. While these results are of interest, it is important to underscore that, due to the relatively small group sizes involved, they should only be considered as preliminary findings that need to be replicated.

Further work is also required to elucidate the mechanisms, differentiate between motor learning and strength training effects, and interpret the potential neuroplastic changes identified from neuroimaging.

ACKNOWLEDGMENTS

The authors thank the volunteers who took part in the study and the radiographers at St. Olavs Hospital who helped with the image acquisition.

GRANTS

The research was funded by Medical Imaging Laboratory (MI-Lab).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: H.S.P., A.K.H., M.S.F., J. Hoff, J. Helgerud, and L.E. conception and design of research; H.S.P., A.K.H., M.S.F., G.M.S., and V.M.I. performed experiments; H.S.P., A.K.H., and L.E. analyzed data; H.S.P., A.K.H., and L.E. interpreted results of experiments; H.S.P. and L.E. prepared figures; H.S.P. drafted manuscript; H.S.P., A.K.H., M.S.F., and L.E. edited and revised manuscript; H.S.P., A.K.H., M.S.F., G.M.S., V.M.I., J. Hoff, J. Helgerud, and L.E. approved final version of manuscript.

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