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Manual activity shapes structure and function in contralateral human motor hand area

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ABSTRACT

From longitudinal voxel-based morphometry (VBM) studies we know that relatively short periods of training can increase regional grey matter volume in trained cortical areas. In 14 right-handed patients with writer's cramp, we employed VBM to test whether suppression (i.e., immobilization) or enhancement (i.e., training) of manual activity lead to opposing changes in grey matter in the contralateral primary motor hand area ($M1_{HAND}$). We additionally used transcranial magnetic stimulation (TMS) to evaluate concurrent changes in regional excitability. Patients were recruited from a clinical trial which was designed to improve handwriting-associated dystonia. Initially the dystonic hand was immobilized for 4 weeks with the intention to reverse faulty plasticity. After immobilization, patients accomplished a motor re-training for 8 weeks. T1-weighted MRIs of the whole brain and single-pulse TMS measurements of the resting motor threshold (RMT) were performed every 4 weeks. Immobilization of the right hand resulted in a relative grey matter decrease in the contralateral left $M1_{HAND}$ along with a decrease in corticomotor excitability as indexed by an increase in RMT. Subsequent training reversed the effects of immobilization, causing an increase in regional grey matter density and excitability of left $M1_{HAND}$. The relative changes in grey matter correlated with the relative shifts in RMT. This prospective within-subject VBM study in task-specific hand dystonia shows that the grey matter density of $M1_{HAND}$ is dynamically shaped by the level of manual activity. This bi-directional structural plasticity is functionally relevant as local grey matter changes are mirrored by changes in regional excitability.

Introduction

Voxel-based morphometry (VBM) of T1-weighted structural magnetic resonance images (MRI) enables unbiased whole-brain analysis of regional changes in human brain structure (Ashburner and Friston, 2000; May and Gaser, 2006). VBM of T1-weighted MRI images has identified areas in the brain where regional grey matter volume is correlated with individual variations in genotype (Binkofski et al., 2007; Pezawas et al., 2004), motor skills (Gaser and Schlaug, 2003), topographical memory (Maguire et al., 2003), or sexual orientation (Ponseti et al., 2007).

Using a within-subject design, VBM of repeated MRI measurements has been employed to map the regional trajectories of brain maturation and aging (Tisserand et al., 2004) or to identify regional alterations in brain structure in a wide range of brain diseases (Daniels et al., 2006; Draganski et al., 2002; May et al., 1999; Nugent et al., 2006). Longitudinal VBM studies also provide a valuable means of monitoring disease progression in the clinical and pre-clinical stage of neurodegenerative diseases (Kassubek et al., 2004; Pennanen et al., 2005; Ramirez-Ruiz et al., 2005; Thieben et al., 2002). In healthy adults, longitudinal morphometric MRI studies showed that intensive training for several days or weeks cause specific increases in cortical grey matter. In these training protocols subjects learn to juggle (Draganski et al., 2004; Driemeyer et al., 2008), learn mirror reading (Ilg et al., 2008), prepare for a medical exam (Draganski et al., 2006), or perform cognitive training (Ceccarelli et al., 2009).

Stimulated by this work, we asked the question whether sustained increase and decrease in cortical activity level result in bi-directional changes in regional cortical grey matter volume. In a prospective VBM study, we recruited 14 patients with writer's cramp, a focal task-specific dystonia during handwriting. These patients participated in

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an interventional study (Zeuner et al., 2008). The interventional protocol consisted of a four-weeks period of immobilization of the affected right hand followed by standardized motor re-training for 8 weeks. Therefore, the study offered the unique opportunity to test the hypothesis that prolonged periods of sensorimotor deprivation (i.e. immobilization) and motor re-training produce opposite changes in regional grey matter in the left primary motor hand area ($M1_{HAND}$). Patients received whole-brain T1-weighted MRI at baseline, immediately after immobilization as well as 4 and 8 weeks into training. We hypothesized that 4 weeks of immobilization would reduce grey matter in the left $M1_{HAND}$ area, whereas subsequent motor re-training would produce a relative increase in regional grey matter.

We additionally performed single-pulse transcranial magnetic stimulation (TMS) to test whether the structural reorganisation of the left $M1_{HAND}$ has a physiological correlate. To this end we measured the resting motor threshold with single-pulse TMS at baseline, immediately after immobilization as well as 4 and 8 weeks into training. We expected that the individual changes in grey matter volume in response to immobilization or training would correlate with the individual changes in corticomotor excitability. Finally, we performed an exploratory analysis to test whether the individual clinical improvement would be reflected in the regional grey matter change in left $M1_{HAND}$.

Materials and methods

Participants

We recruited patients with writer's cramp, a focal dystonia that occurs during writing. Affected patients develop co-contractions of their antagonist muscles during that specific task. Patients also show contractions of muscles that are usually not involved in handwriting (overflow). Dystonic co-contraction and muscular overflow interfere with the highly automated and coordinated movement patterns that generate consecutive strokes during writing. Writer's cramp usually occurs in persons, who have spent much time in writing. As in patients with other types of task specific dystonia, performing a task in a repetitive, stereotyped manner seems to be important. It is currently presumed that a genetic background is important, but environmental factors such as repetitive movements trigger the manifestation of dystonic symptoms (Defazio et al., 2003). In affected patient, a loss of inhibition is hold responsible for the co-contractions and the overflow of activity into muscles not intended for the task.

Patients were enrolled in a therapeutic study which combined immobilization with motor re-training of the affected hand to improve task-specific hand dystonia (Zeuner et al., 2008). 14 out of 21 patients (seven women, mean age: 50.6 years, range: 26 - 71 years) agreed to participate in additional structural MRI in Hamburg (NeuroImageNord, Hamburg) and TMS measurements (TMS laboratory, Kiel) to monitor the effect of immobilization and subsequent sensorimotor training on the structure and function of the $M1_{HAND}$.

Handedness was assessed using the 10-item version of the Edinburgh handedness questionnaire (Oldfield, 1971). All participants had a laterality quotient (range −100 to 100) between 80 and 100 showing that they were consistent right handed. The study had been approved by the local ethics committee. All patients gave written informed consent to the protocol prior to the study. The clinical details of each patient are listed in Table 1. Mean disease duration was 10.1 years at the time of the study, ranging from 2 to 25 years. The severity of dystonia was assessed using two rating scales. The writer's cramp rating scale (WCRS) was used to assess the severity of dystonia (Wissel et al., 1996) during a standardized handwriting task. The higher the total WCRS score the more severe are the dystonic symptoms during handwriting. The Arm Dystonia Disability Scale (ADDS) is a scale that measures how dystonic symptoms interfere with manual daily activities (Fahn, 1989). A score of 100% indicates normal motor function. The lower the ADDS score the stronger is the functional impairment.

Interventional protocol

As described previously, the hand, wrist and lower arm of the affected right upper limb were immobilized for 4 weeks with a splint similar to the one used by Priori et al. (Priori et al., 2001) (CAMP Handgelenksorthese-Halbzirkel, No. 8709, Basko Healthcare, Hamburg, Germany). Patients were allowed to take the splint off for 30 min per day for cleaning and stretching exercises. A temperature log was fixed inside the splint (Kooltrak GmbH, Geisenheim, Germany) and measured local temperature every 30 min over the entire period of immobilization. Temperature measurements enabled us to confirm that patients had actually worn the splint as instructed.

After 4 weeks of immobilization, the dystonic hand was trained for 8 weeks. Patients were randomly assigned to two types of motor re-training. The first group trained writing movements using a pen attached to the bottom of a finger splint (task specific training). The second group trained finger movements using therapeutic putty, but

Table 1
Clinical and experimental details of the patients with writer's cramp. M: male; F: female; ADDS: Arm Dystonia Disability Scale. In the column referring to the type of training "1" refers to task specific training with finger splint and "2" refers to non-task specific training with therapeutic putty. *Grey matter density estimates (values of first eigenvariate determined in left $M1_{HAND}$ volume of interest).

Patients number	Age (years)	Gender	Type of training	Symptom duration (years)	Dystonia scales (baseline)		GM density estimates ($\times 10^{-3}$) at peak [−38 −23 58]	RMT % of max output
					Wissel scale	ADDS	weeks 0/4 /8/12	weeks 0/4/8/12
1	52	M	1	18	14	68.57	292/298/273/307	36/35/34/33
2	63	F	2	25	20	34.29	263/260/230/275	41/36/43/39
3	49	F	1	15	18	17.14	304/297/292/325	37/39/41/36
4	63	M	1	7	10	64.29	369/306/351/359	36/41/36/36
5	34	F	2	3	10	51.42	315/291/327/291	31/36/35/31
6	26	F	1	5	15	72.86	264/274/301/285	30/33/30/28
7	52	M	2	5	6	60.00	317/299/321/316	28/28/28/24
8	71	M	2	5	16	77.14	248/251/260/244	32/33/34/30
9	59	M	2	15	17	8.57	227/202/238/235	31/34/33/28
10	59	F	1	18	13	51.42	189/179/194/188	38/37/36/38
11	46	F	1	3	10	42.86	316/287/290/291	35/36/34/35
12	52	M	2	11	13	72.86	231/262/247/255	27/31/28/28
13	45	M	2	2	2	68.57	315/284/293/298	29/34/29/32
14	38	F	1	10	20	55.71	344/306/336/393	38/47/41/42
Mean (median)	50.6 (52)	7 x M 7 x F	7 x "1" 7 x "2"	10.1 (8.5)	13.1 (13.5)	53.3 (57.9)	285/271/282/290	34/36/34/33

without writing exercises (non-task specific training). The details regarding immobilization and motor re-training as well as the clinical outcome are reported elsewhere (Zeuner et al., 2008). In short, immobilization had no immediate effect on dystonic symptoms, but subsequent training improved task-specific dystonia relative to baseline. Both training modalities were equally effective in reducing dystonic symptoms, showing that training does not need to specifically focus on the task affected by dystonia to be clinically effective.

Structural and functional measurements

T1-weighted whole-brain MRI of brain structure and single-pulse TMS measurements of cortical excitability were prospectively performed every 4 weeks: at baseline (week 0), immediately after 4 weeks of upper limb immobilization (week 4), as well as 4 weeks (week 8) and 8 weeks (week 12) into motor re-training of the affected hand.

Structural magnetic resonance imaging

Three-dimensional structural MRIs of the whole brain were acquired on a 3T Trio system and a standard head coil (Siemens, Erlangen, Germany). We used a T1-weighted FLASH 3D sequence (repetition time [TR] = 15 ms, echo time [TE] = 4.92 ms, flip angle = 25°, 192 slices, slice thickness = 1 mm, matrix: 256 · 256 mm) with an isotropic resolution of 1 × 1 × 1 mm. The structural MRI data sets were pre-processed using the VBM2 toolbox V1.08 (Structural Brain Mapping Group, Department of Psychiatry, University of Jena, http://dbm.neuro.uni-jena.de/vbm/vbm2-for-spm2). The VBM2 toolbox is integrated in the freely available SPM2 software package (Wellcome Dept. of Imaging Neuroscience, Institute of Neurology, UCL, London, www.fil.ion.ucl.ac.uk/spm) running under Matlab 6.5 (MathWorks, Natick, MA). Pre-processing involved spatial normalization of all images to a standardized anatomical space and segmentation of images into the three major tissue types: grey matter, white matter, and cerebrospinal fluid. Structural MRIs were pre-processed according to the "optimized" method (Good et al., 2001). In addition, the SPM2 toolbox uses a Hidden Markov Random Field model to remove isolated voxels of one tissue class and to close holes in a cluster of connected voxels. This procedure reduces the noise level of the segmentation. To optimize the VBM procedure, we created study-specific templates to compensate scanner specific contrast differences and non-uniformities as well as demographic differences of our patient population from those used to build the standard Montreal Neurological Institute (MNI) templates.

The T1-weighted MRIs of each patient were normalized and segmented using the longitudinal data segmentation procedure implemented in the VBM2 toolbox. The longitudinal segmentation procedure derives the full normalization parameters (linear and non-linear components) from the first baseline MRI scan and estimates the rigid body motions (linear only) between the subsequent scans of the same person relative to the baseline scan. The linear and non-linear estimates are then combined to normalize and segment all images from a patient. Spatial smoothing of the normalized grey matter density images (non-modulated data) was performed with a Gaussian kernel of 8 mm full-width-at-half-maximum to remove small misalignments caused by imperfect normalizations and to render the data more Gaussian like.

Voxel based morphometry

The segmented and normalized grey matter images were analyzed using SPM2 by specifying a repeated-measures ANOVA model on a voxel by voxel basis and applied a non-sphericity correction with replications over the factor "subjects" to consider correlations between the repeated measurements. We did not expect any effects depending on the type of training, mainly because the clinical effects were identical and the amount of training, in terms of total time of training, were matched (Zeuner et al., 2008). Furthermore, both types of training engaged the same sensorimotor brain regions involved in fine control of skilled manual movements. Therefore we considered both groups together and restricted our main analysis to the left $M1_{HAND}$ region to maximize the power of the statistical analyses. Our main hypothesis was that immobilization of the right hand and wrist would reduce grey matter regionally in the contralateral $M1_{HAND}$ area and that this deprivation induced atrophy would be reversed in the subsequent training phase. To test this hypothesis we set up a conjunction analysis that took into account two *t*-contrasts which corresponded to two paired single-sided *t*-tests. The first contrast was specified to detect significant decreases in grey matter density from week 0 to week 4, reflecting regional losses in grey matter during immobilization. The second contrast was specified to test increases in regional grey matter density during the training period between week 4 and week 12. By combining these two contrasts, we ensured that the resulting statistical map identified those voxels in the brain where regional grey matter was modified by immobilization and sensorimotor training in an opposite fashion according to our a priori hypothesis.

The left $M1_{HAND}$ region was defined as primary volume of interest (VOI) because of our topographic specific hypothesis expecting morphometric changes to occur in the left $M1_{HAND}$. To test for morphometric changes in this specific region we placed a spherical VOI with a diameter of 8 mm (including 257 voxels) in the left $M1_{HAND}$. The sphere was centred at the stereotactic MNI-coordinates $x = -39$, $y = -24$, $z = 57$ (coordinates were converted from Talairach to MNI space) which corresponds to the location of the left sensorimotor cortex as derived from a recent meta analysis of motor activation studies (Mayka et al., 2006). For all voxels within the VOI, small volume correction (SVC) was applied to correct for multiple comparisons. Outside the left $M1_{HAND}$, area correction for multiple comparisons took into account all voxels in the brain. At the voxel level, statistical threshold was set at $p<0.05$ corrected for multiple comparisons using the familywise error (FWE) method as implemented in SPM. Clusters in which more than 10 contiguous voxels surpass an uncorrected threshold of $p<0.001$ are descriptively reported as statistical trends to inform future studies.

Transcranial magnetic stimulation

Single-pulse TMS was performed at rest using a standard figure-of-eight-shaped coil (Type MC-B70) connected to a MagPro X100 stimulator (MagVenture, Skovlunde, Denmark) (Weyh et al., 2005). The centre of the coil was tangentially placed over the left and right $M1_{HAND}$. We defined the cortical motor threshold at rest (RMT) as neurophysiological marker of interest. The RMT corresponds to the minimal intensity at which a single TMS pulse evokes a contralateral motor response and reflects the trans-synaptic excitability of corticospinal output neurons in $M1_{HAND}$ (Chen et al., 2008).

Several considerations prompted us to choose the RMT rather than other TMS measures of cortical excitability. First, RMT measurements only required subjects to relax without engaging in a motor task. Hence, RMT measures were neither confounded by changes in motor performance in response to the interventions nor affected by individual differences in motivation. Second, the RMT reflects trans-synaptic excitability of the fast conducting corticospinal output neurons in the $M1_{HAND}$ (Amassian et al., 1987; Ziemann, 2004), but the RMT is also sensitive to changes in intrinsic neuronal excitability (Ziemann, 2003). Third, previous TMS work has shown that the RMT is stable across sessions in the absence of any intervention (Boroojerdi et al., 2002). Fourth, previous within-subjects measurements of RMT changes over time were sensitive to changes in motor cortical

excitability induced by limb immobilization in healthy subjects (Facchini et al., 2002) and patients with traumatic fractures (Zanette et al., 2004). Finally, a VBM study based on diffusion-based MRIs showed that the RMT of the hand muscles reflects microstructural properties of the white matter beneath pre-motor and motor cortices which contains cortico-cortical fibres projecting into the $M1_{HAND}$ (Kloppel et al., 2008).

To measure the RMT, we first determined the optimal position for activation of the FDI muscles by moving the coil in 0.5 cm steps around the presumed left $M1_{HAND}$. The coil was placed tangentially to the scalp at a 45 degree angle away to the midline, approximately perpendicular to the central sulcus. Each stimulus had a biphasic configuration with the second phase of the stimulus inducing a posterior-anterior current in the brain (Kammer et al., 2001). The sites where stimuli of slightly suprathreshold intensity consistently produced the largest motor evoked potentials (MEPs) with the steepest negative slope in the contralateral first dorsal interosseus (FDI) muscle (referred to as the "motor hotspot") were marked with a wax pen. The RMT was determined over the motor hotspot in the relaxed muscle and expressed as a percentage of maximum stimulator output. Maximum stimulator output refers to the maximal intensity of stimulation that can be delivered with the MagPro X100 stimulator. The RMT was defined as the minimum stimulus intensity that evoked an MEP in the relaxed contralateral FDI muscle with a peak-to-peak amplitude of more than 50 μV in five out of 10 consecutive trials (Rossini et al., 1999; Tranulis et al., 2006).

Statistical analyses of regional cortical excitability

Repeated-measure ANOVA was performed to test for relative changes in cortical excitability following immobilization and training with the within-subject factor time (4 levels: week 0, week 4, week 8, and week 12) and the RMT as dependent variable. Mauchly's test was used to verify the sphericity assumptions of the statistical model. Statistical threshold was set at $p<0.05$. Depending on a significant main effect of time, we performed planned post-hoc *t*-tests using the same statistical threshold. Our hypothesis specifically predicted an increase in RMT (i.e., decrease in cortical excitability) with immobilization and a decrease in RMT (i.e., increase in cortical excitability) with training. To test this hypothesis we conducted two single-sided paired *t*-tests comparing RMT measurements at baseline to week 4 (immobilization phase) and measurements at week 4 to week 12 (training phase). Additionally we examined the cumulative effects of immobilization and subsequent training on RMT. Here we used a two-sided paired *t*-test, because we had no specific prediction whether and in which direction the opposing excitability changes induced by immobilization and training would outbalance each other.

We computed an additional regression analysis to test whether individual variation of post-interventional excitability changes (as indexed by the RMT) predicted the individual magnitude of grey matter change in $M1_{HAND}$.

The individual RMT changes during consecutive measurements (week 4 minus week 0, week 8 minus week 4, and week 12 minus week 8) were used as an explanatory variable in a SPM regression model. The corresponding images with the grey matter differences were calculated from the smoothed grey matter density images. Voxels with a negative correlation were detected by performing a one-sided *t*-test on the variable containing the RMT differences. As in the previous analysis we expected the main effect around the left $M1_{HAND}$ area. Results are therefore also reported by using a SVC with the same volume used in the previous analysis (Mayka et al., 2006).

To further analyze and visualize the linear relationship between individual changes in grey matter and RMT in the left $M1_{HAND}$, we extracted the first eigenvariate of the grey matter differences from the voxels in the left $M1_{HAND}$ using the SPM VOI tool at a threshold level of $p_{unc}<0.05$. This first eigenvariate captures most of the grey matter variance measured in the left $M1_{HAND}$ volume. In contrast to averaging across all voxels within the VOI, the first eigenvariate is similar to a weighted average. Voxels in the VOI that show a large deviance from the main signal vector are weighted to a lesser extend. Accordingly, with the eigenvariate it is possible to reduce the influence of those voxels that do not follow the main signal trace and might not belong to the $M1_{HAND}$ region. Hence, the first eigenvariate is superior in representing the main signal of the left $M1_{HAND}$. The Pearson's correlation coefficient was calculated to test for a linear relation between the individual RMT measures and the grey matter densities in the left $M1_{HAND}$ area as reflected by the first eigenvariate. The statistical threshold at the voxel level for the regression analyses was set at $p_{FWE}<0.05$. In the left $M1_{HAND}$ area we again restricted the correction for multiple comparisons to the voxels in the left $M1_{HAND}$ area.

Clinical effects of the interventional protocol

A detailed analysis of the interventional protocol on clinical scores over the whole group of participants has already been published (Zeuner et al., 2008). Since only two third of the entire cohort participated in the combined MRI-TMS measurements, we decided to check whether the clinical effects obtained in our subsample were consistent with the previous analysis. Using the WCRS and ADDS as dependent variables, we perform two separate one-factorial repeated measures ANOVAs with four levels for time and checked the sphericity assumptions with Mauchly's test. Depending on a significant main effect of time, post-hoc two-tailed *t*-tests were performed to assess time-dependent changes.

We also computed an exploratory correlation analysis which tested for a linear relationship between relative changes in grey matter density and the clinical presentation of dystonia. The eigenvariate calculated in the $M1_{HAND}$ area was used as representative measure for the regional grey matter change.

Results

Changes in cortical grey matter volume

The changes in manual motor activity as induced by the therapeutic interventions left a structural fingerprint in the grey matter of the contralateral left $M1_{HAND}$ (Fig. 1, Table 2). Immobilization of the right hand and forearm for 4 weeks led to a decrease in regional grey matter volume in the left $M1_{HAND}$. This decrease in grey matter volume was reversed by eight-week period of motor re-training. The conjunction analysis, testing specifically for voxels showing a decrease in grey matter density with immobilization and increase with sensorimotor training, identified a well-defined cluster in the left $M1_{HAND}$. The most consistent modulation by both interventions occurred at MNI coordinate $x=-38$, $y=-22$, $z=59$ ($Z=2.7$, $T=2.85$; $p_{FWE(SVC)}=0.037$) close to the probabilistic location of the $M1_{HAND}$ determined in the meta analysis performed by Mayka et al. (Mayka et al., 2006). This response pattern was somatotopically specific because immobilization and sensorimotor training had no effects on regional grey matter in other parts of the left or right pericentral cortex, even when a liberal threshold of $p_{unc}<0.05$ was applied. The homologous right $M1_{HAND}$ tended to express a pattern of grey matter changes that was inverse to the grey matter changes expressed in left $M1_{HAND}$. The right $M1_{HAND}$ showed a weak trend towards an increase in grey matter volume with immobilization and a decrease in grey matter volume with training ($p_{unc}<0.05$). The structural changes in the right $M1_{HAND}$ are illustrated in the supplementary Fig. 1 which plots the parameter estimates of the voxel showing the peak change in grey matter volume (MNI coordinates $x=29$, $y=-30$, $z=57$).

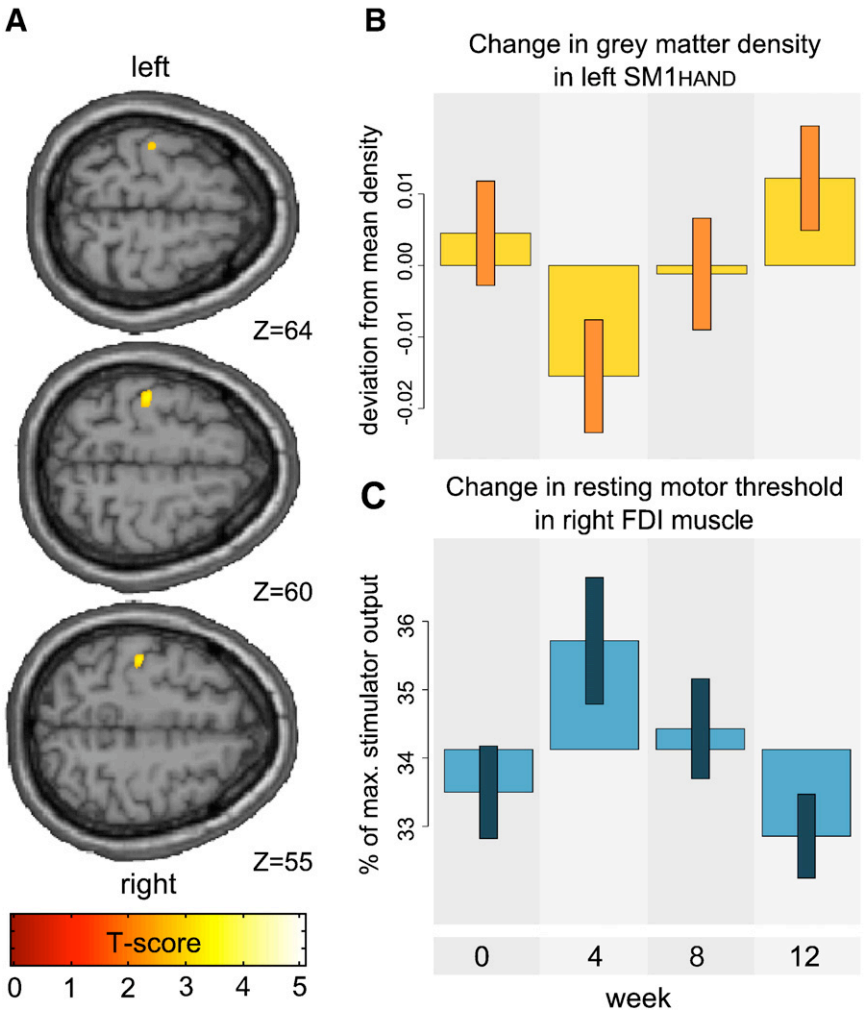

Fig. 1. Panel A. Transversal slices covering the left primary motor hand area ("hand knob"). The statistic parametric map (SPM) gives the voxels showing a relative decrease in regional grey matter density from weeks 0 to 4 (period of immobilization) and an increase in grey matter density from weeks 4 to 12 (period of sensorimotor training). The statistical map has a height threshold of $p_{unc}<0.01$ and a cluster extent threshold of >20 contiguous voxels. Voxels showing a grey matter change are superimposed on the T1-weighted MNI template implemented in SPM2. Panel B. Estimated grey matter density deviations from the overall mean in left $M1_{HAND}$ at MNI coordinates $x, y, z = -38, -22, 59$ (peak voxel). Panel C. Relative changes in resting motor threshold assessed with single-pulse TMS in the right FDI muscle during immobilization and subsequent training. Bars in the columns reflect the deviations from the overall mean. The error bars give the 95% confidence intervals of the mean. Week 0 = baseline; week 4 = after immobilization; week 8 = after 4 weeks of training, week 12 = after 8 weeks of training.

Outside the pre-defined VOI in the $M1_{HAND}$, several left-hemispheric sensorimotor regions displayed a relative decrease in grey matter after immobilization which was subsequently reversed by training, including the anterior insular cortex, postcentral cortex, parietal operculum. The temporal pattern and the magnitude of morphometric changes in these brain regions were comparable to the changes found in left $M1_{HAND}$. However, since these areas had not been defined as VOIs, these morphometric changes did not reach significance after whole-brain correction for multiple comparisons. Nonetheless, we report these regional changes in Table 2 as statistical trends to inform future VBM studies on candidate areas that may show structural plasticity in response to prolonged changes in sensorimotor activity.

Changes in regional cortical excitability

The group data of the RMT measurements are illustrated in Fig. 1C. Test for sphericity was not significant and we therefore did not correct for non-sphericity. Repeated-measures ANOVA revealed a difference in mean RMT measurements among the four measurements ($F_{3,39} = 5.62$, $p = 0.003$). Four weeks of immobilization led to a relative increase in RMT as compared to RMT at baseline ($T_{13} = 2.4$, $p = 0.016$; Fig. 1C). Subsequent training reversed this effect causing a relative decrease in RMT when comparing RMTs at week 12 (i.e., at the end of the training period) to the RMTs at week 4 (i.e., immediately after immobilization; $T_{13} = 4.21$, $p = 0.0005$; Fig. 1C). On average the retraining phase slightly lowered the RMT beyond the pre-interventional level. However, RMTs at the end of the study (week 12) did not differ significantly from RMTs measured at baseline (week 0).

Relation between changes in cortical structure and excitability

A cluster in left $M1_{HAND}$ showed a linear relationship between the relative change in cortical excitability and the relative change in grey matter density (Fig. 2). The stronger the decrease in cortical excitability (as indexed by an increase in RMT) after 4 weeks of immobilization the greater was the relative grey matter increase in the left $M1_{HAND}$. The stronger the increase in cortical excitability (as indexed by a decrease in RMT) after 8 weeks of motor training the greater was the relative grey matter increase in the left $M1_{HAND}$. Peak of the statistical map in the regression analysis was located at voxel at

Table 2
Sensorimotor regions implicated in manual control of the right hand showing a relative change in grey matter density in accordance with our hypothesis (i.e. a relative decrease in grey matter volume from week 0 and 4 due to immobilization and a relative increase in grey matter volume from week 4 to week 12 due to motor training). Stereotactic coordinates (MNI space) and *Z*-scores are given for the voxel showing the strongest effect size in the cluster (regional peak). * significant at $p_{FWE}<0.05$ after small volume correction using a sphere with 8 mm diameter centred on the MNI-coordinates reported in the meta analysis (Mayka et al., 2006).

Brain region	Conjunction analysis					Week 0>Week 4				Week 4<Week 12			
	Stereotactic coordinates			*Z*-score	Cluster extent	Stereotactic coordinates			*Z*-score	Stereotactic coordinates			*Z*-score
	x	*y*	*z*			*x*	*y*	*z*		*x*	*y*	*z*	
Peak voxel of small volume corrected region surviving a threshold of $p_{FWE}<0.05$													
Left primary motor cortex	−38	−22	59	2.70*	22	−38	−22	59	2.70*	−39	−22	59	3.32*
Peak voxels of motor regions outside the $M1_{HAND}$ region surviving a threshold of $p_{uncorrected}<0.001$													
Left postcentral cortex	−27	−41	48	3.75	94	−29	−40	−45	4.25	−27	−41	48	3.75
Right sup. temporal pole	59	4	−11	3.50	26	58	6	−12	3.80	58	5	−11	3.54
Left anterior insula	−43	−8	15	3.30	34	−42	−8	16	3.64	−43	−8	15	3.30
Left parietal operculum	−54	−28	24	3.24	20	−56	−33	29	3.56	−52	−30	22	3.50

MNI coordinates $x=-38$, $y=-23$, $z=58$ ($Z=3.17$; $p_{unc}=0.001$; $p_{FWE(SVC)}=0.019$). This peak location was located only 2.5 mm apart from the probabilistic location of the left $M1_{HAND}$ determined in a meta-analysis (Mayka et al., 2006).

Supplementary correlation analysis (Pearson) of the relationship between cluster changes in grey matter (represented by the first eigenvariate of 241 voxels capturing 84.52% of the variance in the $M1_{HAND}$ cluster) and the RMT showed a negative correlation (cor = −0.42; $T_{40}=2.93$; $p=0.0027$; 95% confidence interval = [−1, −0.18]) which is equivalent to a positive correlation between increases in grey matter density and regional excitability. Separate correlation tests for consecutive time points showed negative correlations for the immobilization period (weeks 0–4; cor = −0.32) and the first 4 weeks of training (weeks 4–8; cor = −0.57), but not for the second phase of training (weeks 8–12, cor = 0.1).

Improvement in hand dystonia

The interventional protocol had consistent effects on the extent of dystonia, resulting in a gradual improvement during the interventional period. Supplementary Fig. 2 illustrates the changes in WCRS and ADDS scores in response to immobilization and subsequent training. Both clinical scores on our subgroup showed no violation of the sphericity assumption and both scores showed significant main effects in time. In agreement with the published results including the entire patient cohort (Zeuner et al., 2008), post-hoc paired *t*-tests revealed a significant decrease of the WCRS score between the examinations of weeks 4 and 12 ($T_{13}=2.29$, $p=0.04$) and over the whole therapy (week 0 to week 12, $T_{13}=4.52$, $p=0.001$). The ADDS score increased from week 0 to week 4 ($T_{13}=2.47$, $p=0.028$), from week 4 to week 12 ($T_{13}=2.61$, $p=0.021$) and over the whole experiment (week 0 to week12, $T_{13}=4.23$, $p=0.001$), but the differences did not reach a significant level when comparing the measurements with the interim re-training examination (week 4 to week 8 and week 8 to week 12).

The overall improvement in task-specific dystonia during handwriting correlated with individual changes in grey matter density from week 0 to week 12. There was a negative correlation between individual changes in grey matter density from week 0 to week 12 and the cumulative changes in WCRS scores induced by immobilization and training from week 0 to week 12 (cor = −0.65; $T_{12}=2.99$; $p=0.011$; 95% confidence interval = [−0.88, −0.19]). This correlation was not found when all consecutive examinations were included in the correlation analysis. No correlation was found between individual changes in ADDS score which indicates general motor disability related to dystonia and changes in grey matter density.

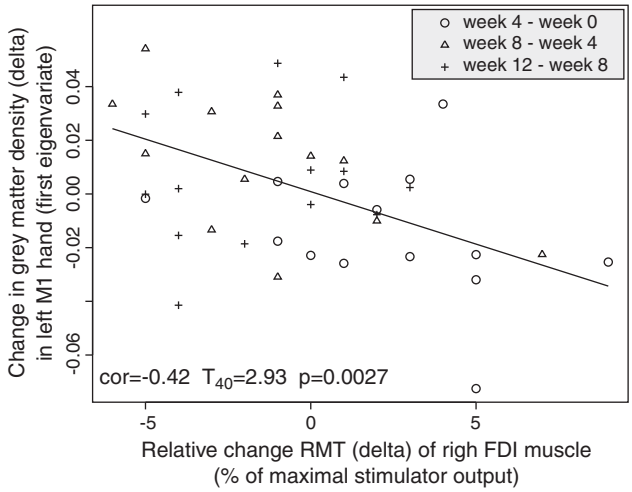

Fig. 2. Scatter plot illustrating the correlation between the relative change in RMT of the right FDI muscle (reflecting a shift in corticomotor excitability of the left $M1_{HAND}$) and relative change in regional grey matter density estimated (1st eigenvariate) in a small volume around the left $SM1_{HAND}$ area at MNI coordinates *x*, *y*, *z* = −39, −24, 57. There is a negative correlation between the two measures showing that a relative increase in motor cortex excitability (as indexed by a decrease in RMT) is associated with a relative increase in regional cortical volume.

Discussion

Combining MRI with TMS in 14 right-handed patients with writer's cramp, we showed that a prolonged suppression (i.e., immobilization) or enhancement (i.e., training) of manual activity induces opposing changes in grey matter in the contralateral $M1_{HAND}$. Immobilization of the right hand led to a relative grey matter decrease along with a decrease in corticomotor excitability as indexed by an increase in RMT. Subsequent training reversed the effects of immobilization, causing an increase in grey matter and excitability in left $M1_{HAND}$. At an individual level, the relative changes in grey matter density correlated with the relative shifts in RMT. We infer that that the grey matter density of $M1_{HAND}$ is dynamically shaped by the level of manual activity. This bi-directional structural plasticity is functionally significant as grey matter changes were paralleled by corresponding changes in regional excitability.

Local reduction in grey matter after immobilization

In patients with writer's cramp, 4 weeks of immobilization of the right dystonic hand and forearm reduced the grey matter volume in contralateral left $M1_{HAND}$. We attribute this “retraction” in cortical volume to the prolonged functional deprivation of the left $M1_{HAND}$

causing a sustained reduction of intracortical neuronal processing. Previous VBM studies on healthy individuals consistently reported regional increases in cortical grey matter volume after several days or weeks of intensive practice (Draganski et al., 2004, 2006; Driemeyer et al., 2008; Ilg et al., 2008; Thomas et al., 2009). These studies indicate that sustained increases in regional neuronal processing during training cause regional expansion of cortical volume in "trained" cortical areas. The present result show that these structural changes are bi-directional as a sustained decrease in regional neuronal activity can produce a "retraction" of cortical volume in deprived cortical areas.

In good agreement with the present results, VBM showed that early visual deprivation results in a relative reduction of grey matter in the visual cortex of blind individuals compared to normally sighted subjects (Pan et al., 2007). The relative grey matter loss correlates with duration of blindness in some occipital visual areas (Ptito et al., 2008). While the VBM results in early blind individuals confirm a tight relationship between prolonged functional deprivation and reduced cortical grey matter volume, the morphometric changes most likely occurred during early life in the developing brain. Expanding the VBM work in the blind, we show that a normal level of neuronal activity is required to prevent localized atrophy of the mature cortex even in later life. Our results have implications for longitudinal VBM studies assessing progression in neurodegenerative diseases. Here a regional grey matter loss may not necessarily indicate a neurodegenerative process, but can to some extent result from a sustained reduction of neuronal activity in "non-used" brain regions.

Reversibility of immobilization-induced grey matter change

The immobilization-induced reduction in grey matter was reversible. The longitudinal VBM-based analysis revealed that the left $M1_{HAND}$ recovered its pre-interventional grey matter volume after eight-weeks of motor training. This bi-directional change in grey matter volume in response to immobilization and training indicates that the mature human primary motor cortex still retains some potential for structural plasticity. A sustained reduction in activity level during immobilization caused the motor cortex to shrink, while enhanced activity levels during training trigger an expansion in cortical volume.

In the present study, all patients were randomly assigned to two different protocols of motor training which were matched for the total time of daily practice. In addition, patients restarted to use their right hand in daily activities. The resumption of skilled dexterous activities and manual training did not only increase the level of neuronal activity, but also required finely tuned patterns of integrated activity in the left $M1_{HAND}$ after the immobilization period. Therefore, we argue that both types of manual activities contributed synergistically to the increase in grey matter volume after immobilization. There was a numerical increase in grey matter volume after 8 weeks of training relative to the baseline measurement before immobilization, but this difference was not significant. Since other groups found significant increases in regional grey matter with prolonged training (Ceccarelli et al., 2009; Draganski et al., 2004, 2006; Driemeyer et al., 2008; Ilg et al., 2008), a continuation of manual training might have resulted in a significant expansion of grey matter volume in left $M1_{HAND}$ above baseline levels.

Parallel changes in corticomotor excitability

The longitudinal electrophysiological and morphometric measurements yielded corresponding changes in corticomotor excitability in left $M1_{HAND}$. Four weeks of limb immobilization increased the cortical motor threshold, indicating a decrease in corticomotor excitability. An increase in RMT was previously reported in healthy subjects in whom the fourth and fifth fingers were immobilized for 4 days (Facchini et al., 2002). The increase in RMT was noted after 3 days of immobilization and returned to baseline within a few days after the end of finger immobilization, but no change in RMT occurred in the primary motor leg area after unilateral immobilization of the ankle joint without peripheral nerve lesion (Liepert et al., 1995). However, the size of the motor cortex area as revealed by TMS mapping diminished in the immobilized tibial anterior muscle, indicating a reduction in corticomotor excitability (Liepert et al., 1995).

In the present study, corticomotor excitability gradually returned back to pre-interventional baseline levels during the 8-week of finger and hand training. The decrease in corticomotor excitability after immobilization correlated with the reduction in grey matter volume. Likewise, the increase in corticomotor excitability with training correlated with the training-related grey matter increase in the left $M1_{HAND}$. In summary, the results showed that the bi-directional changes in grey matter volume were paralleled by bi-directional changes in corticomotor excitability.

A recent morphometric study combined structural with functional MRI to examine the functional significance of activity induced changes in cortical grey matter volume (Ilg et al., 2008). Healthy subjects were studied before and after they practiced mirror reading for 2 weeks. After training, VBM revealed a regional increase in the dorsal occipital cortex close to the region showing peak activation during mirror reading, suggesting a link between practice related activity and the regional increase in grey matter. The current VBM-RMT study extends the findings reported by Ilg et al. (2008) providing a direct link between activity-driven structural changes in $M1_{HAND}$ (as revealed by VBM) and corticomotor function. The correlation between individual shifts in regional excitability and grey matter volume shows that the morphological changes in left $M1_{HAND}$ reliably reflect the impact of immobilization and training on corticomotor function. Our results also show that the morphometric changes as revealed by longitudinal VBM measurements are functionally relevant.

Regional specificity of immobilization-induced grey matter changes

The reduction in grey matter after immobilization was confined to the functionally deprived contralateral left $M1_{HAND}$. No regional reductions in grey matter were found in the face or leg area of the primary motor cortex, even when lowering the threshold to $p<0.05$. In contrast to the left $M1_{HAND}$, the right $M1_{HAND}$ showed a weak trend towards an increase in regional grey matter volume with immobilization. The slight increase in grey matter in the right homologous $M1_{HAND}$ with immobilization of the right ipsilateral hand confirms the somatotopic specificity of the grey matter loss in the deprived contralateral left $M1_{HAND}$. It also supports the notion that the level of neuronal activity has a bi-directional impact on regional grey matter volume. Immobilization of the ipsilateral right hand and forearm forced the patients to use the left non-dominant hand for daily manual skills. We hypothesize that the "forced use" of the left hand raised the level of regional neuronal activity in the right $M1_{HAND}$ resulting in an increase in regional grey matter. Using TMS mapping, analogous effects of forced use on the cortical motor maps have been demonstrated in stroke patients before and after constraint-induced movement therapy (Liepert et al., 1998, 2000).

The left postcentral cortex, anterior insula and parietal operculum showed a change in local grey matter density with immobilization and training that was comparable in magnitude and sign to the changes found in the left $M1_{HAND}$ region (Table 2). These brain regions are involved in processing and integrating sensory input from the contralateral hand (Eickhoff et al.; Fink et al., 1997). These analogous changes in grey matter density outside the left $M1_{HAND}$ indicate that the pericentral cortex may not be the only brain area where regional structure is shaped by the level of manual sensorimotor activity.

Immobilization and training also seem to influence grey matter structure in other cortical regions within the sensorimotor network. However, these changes were located outside our pre-defined region of interest, and did not survive correction for multiple comparisons. Therefore these findings are not discussed further, but they might be useful to inform future morphometric studies.

Link between clinical improvement and motorcortical grey matter change

The overall improvement in task-specific dystonia during handwriting as reflected by a decrease in WCRS score from week 0 to week 12 correlated with individual increases in grey matter density from week 0 to week 12. This finding is in good agreement with the notion that regional increases in cortical grey matter are associated with functional capability. However, the relative change in grey matter induced by each intervention (i.e., immobilization or training) did not correlate with individual grey matter changes in $M1_{HAND}$ when comparing consecutive MRI measurements. In other words, the symptom–structure relation only held for the cumulative effect of both interventions, whereas the changes in cortical structure related to each intervention alone reflected the manipulation of the activity level rather than their impact on the clinical status. We argue that relative changes in regional grey matter volume may be related to prolonged changes in the cortical activity levels as well as to changes in the clinical status caused by the interventional protocol. This should be born in mind when interpreting the longitudinal effects of therapeutic interventions on regional cortical brain structure.

Limitations of the study

Our study has some limitations that need to be discussed. First, the study only included patients with writer's cramp. We already encountered problems in recruiting a sufficient number of patients in our study because many patients did not wish to immobilize their limb for 4 weeks. This was in part because of the substantial limitation of daily activities during immobilization, but also because a four-week period of immobilization was incompatible with their current job conditions. One patient experienced a major adverse event during immobilization because immobilization led to an acute exacerbation of a latent carpal tunnel syndrome (Zeuner et al., 2008). Practical and ethical considerations lead us to refrain from including a group of healthy age-matched controls.

Since cortical sensorimotor plasticity was found to be impaired (Hallett, 2006; Quartarone et al., 2006), it is possible that the structural and functional responses of the $M1_{HAND}$ to immobilization and manual training may be different in healthy individuals without dystonia. While healthy individuals may well show a difference in the magnitude of structural changes relative to patients with writer's cramp, we expect activity related structural changes in the same direction, namely a decrease in grey matter density with limb immobilization and an increase with motor training.

Second, we can still only speculate about the exact cellular mechanisms that are driving the changes. The associated bi-directional changes in corticomotor excitability emphasise the functional relevance of grey matter changes in response to interventions that suppress or enhance the regional activity level in the human cortex. Activity dependent loss or formation of cortical synapses represents a candidate mechanism behind the observed bi-directional changes in grey matter and corticomotor excitability in the $M1_{HAND}$ (Grutzendler et al., 2002; Trachtenberg et al., 2002). A prolonged shift in the level of neuronal activity in the $M1_{HAND}$ may reduce (in the case of immobilization) or increase (in the case of motor learning) the total number of excitatory synapses of intracortical axons projecting onto the corticospinal output neurons, thereby producing bi-directional shifts in the RMT. In parallel, the activity driven change in the number of synapses and dendritic spines may account for the bi-directional changes in grey matter volume (Huber et al., 2006). Alternative mechanisms include activity driven changes in spine morphology and perisynaptic astroglia (Bourne and Harris, 2008; Harms et al., 2008; Kleim et al., 2007) or activity driven changes in capillary density or diameter (Anderson et al., 1994). These structural changes may contribute to the strengthening (LTP) and weakening (LTD) of cortical synapses and resulting changes in cortical excitability. Finally, it cannot be excluded that changes in excitability at the spinal level contributed to the observed changes in RMT, as RMT is influenced by both, the cortical and spinal level of excitability.

Since the data were acquired in a clinical trail, the sequence of interventions was pre-defined by the study protocol. Therefore the order of interventions was fixed with immobilization preceding training. To fully access the directionality specificity of this plasticity and to explore possible hysteresis effects it would be necessary to conduct a parallel study in another group of patients who receive a reversed order of intervention with sensorimotor training preceding immobilization. A limitation of the study was that we did not use a double-blinded study design. This might have biased the examiners when measuring the RMT. However, examiners had no a priori information regarding the previous RMT measurements and no knowledge about the specific research hypotheses. Furthermore, a bias in the RMT measurements should have adversely affected the correlation between the inter-individual variations in RMT and grey matter changes.

Using single-pulse or paired-pulse TMS a wide variety of excitability measures can be obtained including active motor threshold, the stimulus-response curve, cortical silent period or paired-pulse intracortical inhibition and facilitation (Hallett, 2000). In this study, we only focused on RMT although it would have been interesting to include additional excitability measures. Including a range of excitability measures would have required adjustments for multiple comparisons reducing the sensitivity of statistical testing for our primary measure of interest, the RMT. However, future studies will need to address how other measures of motor cortex excitability are changed by immobilization and motor retraining and how such changes translate into alterations of regional grey matter volume in $M1_{HAND}$.

We are aware that the observed changes in RMT may also be caused by changes on the spinal level even if gray matter changes in M1 were evident.

An increasing body of evidence suggests that activity dependent structural plasticity is also regionally expressed in white matter pathways (Fields, 2008) and that diffusion based MRI is a sensitive means to trace regional changes in white matter structure associated with prolonged changes in regional brain activity (Johansen-Berg, 2009). For example, the posterior intraparietal sulcus showed an increase in fractional anisotropy (FA) after healthy individuals had learned a complex visuo-motor skill (Scholz et al., 2009). Likewise, individuals who were trained on working memory for 2 months showed regional increases in FA were found in the intraparietal sulcus, corpus callosum and at the border between the frontal and parietal lobe (Takeuchi et al.). Interestingly, increased FA values in white matter were also found in fibre tracts connecting the primary sensorimotor areas with subcortical structures in writer's cramp (Delmaire et al., 2009). All these findings underscore the impact of training on human brain structure in the adult human brain. Since this study exclusively focussed on cortical grey matter density, we have no information how immobilization and training gave rise to the subcortical white matter underlying the $M1_{HAND}$. One might expect similar increases in FA in sub-motor cortical white matter. The relationship between regional grey and white matter changes caused by prolonged changes in regional activity levels need to be addressed in future studies.

In summary, we show that experimental manipulations that induce prolonged decreases or increases in cortical neuronal activity

can trigger a retraction or expansion of regional grey matter volume. This activity-driven bi-directional structural plasticity can be traced *in vivo* with high-resolution structural MRIs of the human brain. The corresponding changes in neuronal excitability indicate that the morphometric correlate of structural plasticity is functionally relevant.

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