Changes in cortical, cerebellar and basal ganglia representation after comprehensive long term unilateral hand motor training

A.D. Walz1*, K. Doppl1*, E. Kaza1, S. Roschka2, T. Platz2, M. Lotze1

1Functional Imaging; Institute for Diagnostic Radiology and Neuroradiology, Ernst Moritz Arndt University of Greifswald, Germany

2BDH-Klinik Greifswald, Neurorehabilitation Centre and Spinal Cord Injury Unit, Greifswald, Functional Imaging Unit; Center for Diagnostic Radiology and Neuroradiology; University of Greifswald, Germany

*both authors contributed equally

Abstract

We were interested in motor performance gain after unilateral hand motor training and associated changes of cerebral and cerebellar movement representation tested with functional magnetic resonance imaging (fMRI) before and after training. Therefore, we trained the left hand of strongly right-handed healthy participants with a comprehensive training (arm ability training, AAT) over two weeks. Motor performance was tested for the trained and non-trained hand before and after the training period. Functional imaging was performed for the trained and the non-trained hand separately and comprised force modulation with the fist, sequential finger movements and a fast writing task. After the training period the performance gain of tapping movements was comparable for both hand sides, whereas the motor performance for writing showed a higher training effect for the trained hand. fMRI showed a reduction of activation in supplementary motor, dorsolateral prefrontal cortex, parietal cortical areas and lateral cerebellar areas during sequential finger movements over time. During left hand writing lateral cerebellar hemisphere also showed reduced activation, while activation of the anterior cerebellar hemisphere was increased. An initially high anterior cerebellar activation magnitude was a predictive value for high training outcome of finger tapping and visual guided movements. During the force modulation task we found increased activation in the striate.

Overall, a comprehensive long-term training of the less skillful hand in healthy participants resulted in relevant motor performance improvements, as well as an intermanual learning transfer differently pronounced for the type of movement tested. Whereas cortical motor area activation decreased over time, cerebellar anterior hemisphere and striatum activity seem to represent increasing resources after long-term motor training.
Introduction

Most of motor training performed during life time can be classified as long-term training procedures. However, imaging studies investigating effects of long-term motor training are rare. Long-term motor training procedures are especially important for regaining motor skills after damage of the central nervous system. Knowledge about processes going on during long-term training in healthy individuals might help to understand changes in patients after brain damage.

In general, motor skill learning is indicated by performance improvement of the sensorimotor system and changes in associated cerebral and cerebellar activation maps. With long-term practice performance becomes more precise and automatic including the suppression of associated movements of the other hand during unilateral movement execution [1]. Such behavioral modifications have been found to be reflected by structural and functional changes in the brain. Especially professional instrumentalists, who undergo extremely intensive long-term motor training, showed reduced functional representation sites for motor and sensorimotor processing in comparison to less trained subjects. During the performance of sequential finger movements this has been particularly evident for the ipsilateral primary motor cortex (iM1), ipsilateral primary somatosensory cortex (iS1), bilateral supplementary motor area (SMA), bilateral dorsal premotor cortex (dPMC), and bilateral superior parietal lobe (SPL) [2-5].

Decreased cognitive effort after long-term motor training results in a decreased activation magnitude in representation sites for processing (e.g. working memory [6]). The prefrontal cortex is involved during acquisition of new motor skills, but not during automated performance [7]. The lateral prefrontal cortex is involved in the support of cognitive control operations [8,9]. However, processing of cognitive aspects is not restricted on cortical areas, but is also seen in latero-posterior cerebellar hemispheres [10]. Other loops in the anterior-medial cerebellar hemisphere are associated with early motor learning, especially with sensorimotor integration [11]. In addition, automatization of movement performance is mirrored in changes of striatal activation [12]. Depending on the stage of motor skill acquisition several studies reported increased activation after finger sequence training in the basal ganglia [13,14], whereas others found a decrease in basal ganglia structures, such as the putamen (unilateral) and caudate (bilateral movements) during sequence learning over several days with 3 hours of overall training time [9]. It has been suggested that the putamen and pallidum are primarily involved in performance of motor skills, whereas the caudate is involved in acquiring the motor sequence knowledge [15]. Using incidental learning, Grafton and colleagues described increases in putamen, but decreases in the caudate with skill development [16]. With respect to training of memory tasks a very detailed model integrating striatal activation increase has been proposed recently [17]. The complex changes in the interactions of motor areas during training have been integrated in different models. Doyon and colleagues [18] assumed a distinct contribution of the cortico-striatal and cortico-cerebellar systems to different motor skill learning phases in dependence on the type of learning, i.e. motor sequence learning or sensorimotor adaptation. Robertson suggested that both paradigms activate the striatum and cerebellum, but that sensorimotor paradigms are more dependent on cortical motor areas while motor sequence learning is more associated with prefrontal activation [12]. By applying a statistical metaanalysis approach it has been shown that activity in the basal ganglia and cerebellum might be more frequently associated with sensorimotor tasks, while activity in cortical structures (SMA, dPMC, SPL) and the thalamus seems to be stronger for serial response time tasks. Furthermore the left dorsal premotor cortex is suggested as a key structure in the network of brain areas that underlie motor learning [19].
Hikosaka and colleagues [20] developed a model of motor sequence learning based on behavioral and physiological experiments with monkeys and humans using sequential button-press tasks that require very limited sensorimotor feedback processing. They assume that during the course of sequential skill acquisition two representations of a motor sequence (spatial and motor) are acquired independently parallel by two sets of cortex-basal ganglia and cortex-cerebellum loop circuits. The authors hypothesize that the acquisition of an effector-unspecific representation in spatial coordinates is predominant in the early stage of learning. This early stage includes rapid changes in areas such as parietal and premotor cortical regions, the caudate, and associative cerebellar regions. In contrast, when the performance becomes automated after long-term practice, the procedure is thought to be acquired predominantly as an effector-specific sequence involving predominantly M1, dPMC, SMA, the putamen, and the cerebellum. In motor adaptation, the cortico-striatal-cerebellar systems contribute to motor learning in another manner than in sequential motor learning, namely with a predominant role of the cerebellum especially in the late phase of learning [18,21].

Motor skill learning involves an effector-dependent and an effector-independent component [22]. It does not only comprise acquisition of the trained motor patterns but also the ability to transfer what has been learned to new conditions [23]. Positive effects of a specific training have been demonstrated for healthy participants not only on the trained but also on the non-trained extremity [24-27] as well as for performing another not specifically trained motor task with the same extremity [28]. Furthermore, similar findings have also been reported after stroke resulting in mild arm paresis for learning transfer on non-trained tasks [29,30] as well as from the healthy to the paretic hand [31]. In fact, training time in a range of weeks to months is essential to understand the effects observed in motor rehabilitation.

Nevertheless imaging-studies investigating longer periods of motor training are rare. Therefore we applied a comprehensive long-term motor training (Arm Ability Training, AAT [29,32]) for the non-dominant hand over two weeks to investigate behavioral and imaging effects for the trained and non-trained hand in healthy adults. We selected the non-dominant hand since we intended to apply a training procedure developed for stroke patients and long-term training of the dominant hand might show a ceiling effect. We were interested in (1) motor efficiency changes for the trained arm characterized by less cortical activation in association with increased performance, (2) intermanual learning transfer on the non-trained arm, and (3) cortical, striatal and cerebellar activity changes associated with these processes.

Materials and Methods

Participants

Fifteen subjects (mean age ± standard deviation: 24 ± 3.7 years; 6 women) participated in this study. All were strongly right-handed (mean laterality quotient (LQ) in the Edinburgh Handedness Test [33]: 93.53 ± 5.5; range: 88-100). All participants were healthy, without any neurologic or cardio-vascular disease and were not taking any regular medication. They were recruited by an announcement posted at the university. Full written consent was obtained from all participants in accordance to the Declaration of Helsinki. The study was approved by the ethics committee of the Medical Faculty, University of Greifswald.

Training

All participants underwent a two-week arm ability training (AAT) for their left, nondonominant hand for one hour per day (11 training days). The AAT had previously been developed for
patients after cerebral damage such as stroke [32]. It consists of eight motor tasks: aiming (Ai), tapping (Ta), crossing circles (Cr), turning coins (Tu), labyrinth (La), bolts and nuts (Bo), placing small objects (Ps), placing large objects (Pl) [32]. The AAT targets different sensorimotor abilities such as aiming (i.e. ability to perform quick goal-orientated movements), arm-hand steadiness (i.e. ability to keep the hand or arm steady), wrist-finger speed (i.e. ability to make fast isolated alternating movements of wrist and fingers), finger dexterity (i.e. ability to manipulate small objects), manual dexterity (i.e. ability to grip and manipulate large objects with hands and arms) and visuomotor tracking (i.e. ability to move precisely under continuous visual control) [29]. Time needed for the execution of each of the eight trained tasks was recorded and fed back graphically on a computer. Improved performance was indicated by reduced performance time, while the accuracy demands of the tasks were kept constant. All participants performed the training in the same standardized manner with two runs of task performance in a fixed sequence twice a day [27].

**Motor assessment**

Before and after the training period maximum grip force [bar] was assessed with a Vigorimeter (Gebrüder Martin GmbH & Co. KG, Tuttlingen, Germany) respectively three times for each hand and averaged (averaged maximum grip force; AMGF). Furthermore, we investigated motor performance (time needed in seconds) of both hand sides in a pseudorandom order for all eight tasks of the AAT. In order to determine the effects of training (left hand) as well as the intermanual transfer effects (right hand), we calculated a two-way repeated measures ANOVA with these values for each hand separately including the factors TASK (8 AAT-tasks: Ai; Ta; Cr; Tu; La; Bo; Ps; Pl) and TIME (pre-AAT; post AAT). In case the assumption of sphericity was violated, Greenhouse-Geisser epsilon adjusted p-values were used. Subunits of the motor outcome measures tested before and after training were combined to the following functional units: sequential finger movements (AAT-Ta), grip force (AMGF) and visually guided movements (AAT-Ai, AAT-Cr, and AAT-La). Subsequently, values of percentual improvement were calculated for these three functional units for each hand separately. For visually guided movements, a percentual improvement value was calculated by averaging performance across the corresponding tasks. We calculated a two-way repeated measures ANOVA with the percentual improvement values including the factor HAND (left; right) and TEST (sequential finger movements, maximum grip force, visually guided movements), followed by post-hoc tests Holm-Bonferroni corrected for multiple comparisons to assess training-associated changes over time.

**fMRI experimental design**

Participants lay supine on the scanner bed during task execution. Three different tasks for each hand were performed in a blocked design, consisting of 5 blocks rest alternating with 4 blocks task performance. During all conditions task performance and rest were indicated by “off” and “on” visual cues. These signals were presented via video projection controlled by the Presentation software (Neurobehavioral Systems. Albany, NY, USA) and triggered by the scanner. All tasks were trained for 2 minutes each prior to scanning to ensure proper performance and all motor tasks were continuously monitored during fMRI-scanning. The following tasks were performed for each hand (hand side and task were tested in a pseudorandom order):

- Fist clenching: participants performed a dynamic hand grip task (fist clench). The rate of the hand grip as well as the amplitude of grip force was recorded using a pneumatic rubber ball system [34]. Prior to each scanning session the participant was asked to grip the ball with
maximum force to generate a maximum voluntary contraction (MVC) for 10 times. Then the participant was trained to press the ball with target force 33% MVC and target rate 1 Hz. During scanning the required rate of hand-grip was indicated visually by a circle displayed at the center of the screen. During training and scanning the recorded amplitude of exerted grip force (extending bar) as well as the target amplitude (red line) were fed back via a screen and a beamer in the scanner room and could be observed via a custom made Siemens headcoil mirror. For analysis purposes, the fMRI fist clenching task was assigned to the functional unit grip force.

- Finger sequence: participants performed a sequential finger-tapping task of 12 visually presented numbers indicating button presses on a pad with digits 2, 3, 4, and 5 (corresponding to the index, middle, ring, and small finger, respectively) at a given frequency of 1Hz (visually paced). This task was balanced for motor performance between the pre and post measurements. The task was performed on specially constructed MR-suitable keyboards with physiologically ordered buttons of 1.5 cm in diameter, connected via an optic-fiber cable to a computer outside of the scanner room, and recorded with Presentation (version 13.0; Neurobehavioral Systems, Albany, 2009). The task required evenly fast, isolated sequential finger movements and visuomotor integration. Performance speed was kept constant. Numerical sequence and clock generator were displayed on the same, single screen. For sequential finger movements, the mirror-ordered sequence was used for tapping with the non-trained arm. fMRI finger sequence was assigned to the functional unit sequential finger movements.

- Writing: participants were instructed to copy 10 conjunctions (e.g. “and”, “or”, “between”) arranged in two columns (5 words each) at the center of a sheet of paper (210×297 mm) with a pencil in cursive handwriting. The sheet was affixed on a desk with an angled board that was positioned above the individual’s abdomen, so it was possible to write in supine position. The participants were asked to start copying at the top of the column next to their currently writing hand in a fast but comfortable velocity and in readable words. This task was therefore balanced for the cognitive demand but it enabled investigation of training induced changes in motor performance (number of words) in the fMRI-scanner. Subjects wrote on lines provided directly under the specified words. If they finished copying the conjunctions of the first column before the active block ended, they continued copying on the top of the second one. There were four sheets comprising different conjunctions each with the same number of letters, and presented in a pseudorandomly counterbalanced order during the active blocks. The assistant was standing next to the scanner during the fMRI investigation, changing the paper sheets between the different scanning tasks. Visual contact with the paper sheets for reading and writing was enabled by a double mirror system attached to the head coil. The participants’ upper arms were supported by cushions to restrict arm movements during writing, and the desk position was individually adjusted with respect to the arm length. The performance measure was the number of copied letters averaged over the four trials. The task required finger dexterity, aiming, visuomotor tracking, and arm-hand steadiness. The particular feature of the task was that writing with the right hand has already been highly overlearned. fMRI writing was assigned to the functional unit visually guided movements.

fMRI-data of one participant was not saved. Therefore, the following analysis was completed with the data of the remaining 14 participants. Paired t-tests between pre and post measurement for the respective outcome measures of each fMRI task performed with the left and right hands were calculated. Additionally, we calculated differences between the performances of the left and the right hand with paired t-tests corrected for multiple comparisons by the Bonferroni-Holm procedure. Furthermore, effort was rated for task performance (0-10 numerical rating scale (NRS); 0 = “no effort”; 10 = “maximum effort”). Wilcoxon Signed Rank Tests were
calculated to compare the medians of the NRS values in order to assess possible differences in effort made on the performance of each task between pre- and post-measurement (separately for each hand).

**fMRI data acquisition**

MRI data were collected before and after two weeks of arm ability training using a 3 Tesla MRI scanner (Magnetom Verio, Siemens, Erlangen, Germany) equipped with a 32-channel head-coil. Field homogeneity was optimized prior to each session using a shimming sequence. For each participant, a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence was recorded as a structural dataset, with parameters: repetition time (TR) 1.69 sec; echo time (TE) 2.52 ms; 176 sagittal slices; voxel size 1×1×1 mm³; 2 times generalized autocalibrating partially parallel acquisition (GRAPPA) acceleration. During the performance of each, 90 volumes with 34 slices each (3 mm thick, 1 mm gap) were acquired in the transverse direction, parallel to the AC-PC-line, using echo-planar imaging (EPI; TR 2000 ms, TE 30 ms, flip angle 90°, field of view (FOV) 192x192 mm², matrix size 64x64, voxel size 3×3×3 mm³). The first two volumes of each session were discarded to allow for T1 equilibration effects. Each block lasted 3 minutes, active and resting blocks alternating every 20 seconds. Thirty-four phase and magnitude images were acquired in the same FOV by a gradient echo sequence (flip angle 60°; FOV 192x192; slice thickness 3 mm; TR 488 msec; TE1 4.92 msec; TE2 7.38 msec) to calculate a fieldmap aiming at correcting geometric distortions in the EPI images [35].

**fMRI data analysis**

Data were analyzed using SPM8 (Wellcome Department of Cognitive Neuroscience, London, UK) running on Matlab version 7.8 (MathWorks Inc; Natick, MA, USA). Unwarping of geometrically distorted EPIs was performed in the phase encoding direction using the FieldMap Toolbox available for SPM8. Each individual scan was realigned to the first scan to correct for movement artifacts. The realigned and unwarped EPIs were coregistered to the T1-weighted anatomical image. For normalization, the coregistered T1 image was segmented, normalized to the Montreal Neurological Institute (MNI) template, and the EPIs were resliced at 3×3×3 mm³. The resulting images were smoothed with a 9×9×9 mm³ [full-width at half maximum (FWHM)] Gaussian Kernel filter to increase the signal-to-noise ratio. A temporal high-pass filter (128 s) was applied to remove slow signal drifts. Individual statistical maps (fixed effect) of the main conditions and the pre-post comparisons were evaluated for each participant using the general linear model. For an fMRI group analysis across all subjects, the corresponding contrast images computed on the single subject level were compared in a full factorial general linear model (GLM) random effects analysis (separately for each hand) with the within-subject factors TIME (levels pre-AAT, post-AAT) and TASK (fist clenching, finger sequence, writing), which accounts for the variance between participants. We obtained a significant TIME x TASK interaction. Thus post hoc contrasts were carried out to investigate significant changes in brain activity between the pre- and the post- measurements of each task separately (pre minus post; post minus pre).

In addition, we performed a correlation analysis between the degree of improvement in the functional units mentioned above with the imaging data of the corresponding motor paradigm by calculating whole brain regression analysis. Therefore the contrast images calculated for the pre-post comparison of the respective motor paradigm on the single subject level were entered.
into a SPM multiple regression analysis with the percent improvement values of the corresponding motor task as covariate.

Activation maps for main effects and conjunctions (Figure 3) were thresholded with p<0.05, FWE– corrected over the whole brain. For the investigation of training effects and for the correlation analysis, we used a regions of interest (ROIs) approach and corrected for multiple comparisons within ROIs with p < 0.05, FWE–corrected. We selected the following ROIs in both hemispheres: primary somatosensory cortex (S1), primary motor cortex (M1), supplementary motor area (SMA), the premotor cortex (dorsal: dPMC; ventral: vPMC), superior parietal lobe (SPL; Brodmann area (BA) 5 and BA 7), inferior parietal lobe (IPL), basal ganglia (putamen, pallidum, caudate), and cerebellum. We distinguished between cerebellar vermal and hemispheric activity by using the appropriate masks [36]. For the differentiation of the PMC and the SMA, which are cytoarchitecturally similar, we performed a spatial differentiation: The PMC was defined as the BA 6 lateral to the superior frontal sulcus of the MNI-template (-30 < x > 30) and reached to z = 50 at its inferior border. Spatial assignment of significant brain areas was conducted with the SPM Anatomy Toolbox Version 1.7 [37] and, if regions were not defined by ANATOMY, using anatomical masks from the Automated Anatomical Labeling software [38].

Results

Behavioral outcome measures

A two-way repeated measures ANOVA with the factors AAT tasks pre and post training for performing with the left hand (trainings effects) showed significant effects for the factor TASK (fist clenching, finger sequence, writing; F(7,98) = 22.27; p < 0.001) and TIME (pre-AAT, post-AAT; F(1,14) = 592.26; p < 0.001) as well as a significant TASK x TIME interaction (F(7,98) = 6.56; p < 0.001).

A similar ANOVA of the AAT tasks pre and post training for performing with the right hand (intermanual transfer effects) showed significant effects for the factor TASK (F(7,98) = 19.84; p < 0.001) and TIME (F(1,14) = 152.96; p < 0.001) but no significant TASK x TIME interaction (F(3,11,98) = 1.97; n.s.). Figure 1 shows the percentual improvement for all eight AAT tasks over time.

A two-way repeated measures ANOVA carried out for the percentual improvement of AAT tasks from pre to post differentiated into the three functional units described above revealed significant effects for the factor HAND (F(1,14) = 18.49; p < 0.001) and TEST (F(1,28) = 64.48; p< 0.001) and a significant HAND x TEST interaction (F(1,28) = 11.73; p < 0.001).

One-sample t-tests (Bonferroni corrected for multiple comparisons) showed significant improvements of sequential finger movements for both hands (left hand: t(14) = 12.11; pc < 0.001; right hand: t(14) = 10.13; pc < 0.001) as well as for visually guided movements (left hand: t(14) = 16.84; pc < 0.001; right hand: t(14) = 14.38; pc < 0.001) but no significant improvements in grip force (left hand: t(14) = 0.75; n.s.; right hand: t(14) = 1.5; n.s.). Paired t-tests showed no significant differences between improvements of the left and the right hand in sequential finger movements (t(14) = 2.12; n.s.), but a significant larger improvement in visually guided movements performed with the left hand than performed with the right hand (t(14) = 8.07; pc < 0.001; Figure 2).
Performance during fMRI-scanning

Overall, subjects’ amplitude and frequency during fist clenching between pre- and post-measurements were comparable for both hands (paired t-tests; amplitude/right hand: \(t(13) = -2.04\), n.s.; amplitude/left hand: \(t(13) = -0.97\), n.s.; frequency/right hand: \(t(13) = -0.5\), n.s.; frequency/left hand: \(t(13) = -0.56\), n.s.).

The same was true for the frequency during sequential finger movements between pre- and post-measurements (paired t-tests between pre- and post-measurements; frequency: right hand: \(t(10) = -0.75\), n.s.; left hand: \(t(10) = -1.53\), n.s.), due to the given paradigm with visually presented finger onsets in frequency of 1 Hz.

For writing, the analysis of the number of written letters revealed no differences between the pre- and post-measurement for the right hand (post - pre; paired t-test; \(t(13) = 2.00\), n.s.) but a significant increase in performance with the left hand (paired t-test; \(t(13) = 2.82\), pc < 0.05). However, during both time units writing performance was better when performed with the right hand (pre: \(t(13) = 11.13\), pc \(\leq\) 0.001; post: \(t(13) = 9.33\), pc < 0.001).

The effort indicated during performance (numeric rating scale; NRS) was comparable for all tasks between pre- and post-measurements for both, the left hand (Wilcoxon Signed Rank Tests: fist clenching: \(Z = -0.95\), n.s.; finger sequence: \(Z = -0.48\), n.s.; writing: \(Z = -0.94\), n.s.) and the right hand (Wilcoxon Signed Rank Tests: fist clenching: \(Z = -0.68\), n.s.; finger sequence: \(Z = -0.84\), n.s.; writing: \(Z = -1.12\), n.s.). When analyzing the button presses during the performance of the finger sequencing task, a comparable frequency was confirmed for the pre- and the post-measurement (paired t-tests between pre- and post-measurements; frequency: right hand: \(t(10) = -0.75\), n.s.; left hand: \(t(10) = -1.53\), n.s.). This indicates that participants followed the given protocol.

fMRI findings

Conjunction:

A conjunction analysis was performed to describe activation sites common for movements with both hands over time. These areas might be the candidates for a transfer of performance gain from the trained to the non-trained hand side. For the fist clenching task we observed left M1, dPMC and vPMC, bilateral SMA and SPL, left IPL, bilateral V5 and right anterior cerebellar hemisphere-activation. For the finger sequence task we found a bilaterally symmetric activation pattern in the SMA, dPMC, vPMC (reaching into BA 44), M1 and S1. In contrast, writing showed an activation pattern for the same areas lateralized to the dominant left cerebral hemisphere. On the right cortical hemisphere only dPMC, vPMC reaching into BA 44 and SPL were activated. This indicates very specific lateralization especially by comparing the sequential finger to the writing movements. Figure 3 and Table 1 depict the results of the conjunction analysis.

Main effects:

For the fist clenching task we found activation in contralateral M1, S1, PMC, bilateral SMA and ipsilateral anterior cerebellar hemisphere for each hand side. Only the left hand clenching involved the ipsilateral SPL. The main effect for the pre and post conditions for the finger sequence and the writing task is depicted for the cortical, basal ganglia and cerebellar level in the Supplementary Figure 1. Finger sequence showed a quite homogeneous lateralization pattern for both hand sides. Cortical representation showed low lateralization for both hands,
the basal ganglia were activated and during the pre-measurement the cerebellum showed bilateral activation, too. Writing involved more functional activation when performed with the dominant right hand.

On the cortical level, there was a strong lateralization to the dominant left hemisphere for writing with either hand. Furthermore, the basal ganglia were only activated during writing with the right hand. Only left hand writing showed a recruitment of the contralateral inferior posterior cerebellar hemisphere.

**Effects over time:**

For the comparisons pre minus post (decrease of activation during training) we observed distinctive reduced activation for the motor tasks specific for the trained hand (Figure 4, Table 2). Fist clenching with the left hand did not show relevant activation decrease over time. Left finger sequence performance showed an activation decrease in the dorsolateral prefrontal cortex (dIPFC, BA 8), ipsilateral S1 (BA1, 2, 3), ipsilateral superior parietal lobe (SPL; BA 5 and BA 7), ipsilateral inferior parietal sulcus (IPS, BA 40), and bilateral SMA (BA 6). During writing reduced activation was observed in the ipsilateral posterior cerebellar hemisphere (Larsell H VIIA; crus 1).

For the post minus pre comparison fist clenching showed increased contralateral pallidum and bilateral putamen activation after training. Left hand writing showed an effect for post minus pre in the left anterior cerebellar hemisphere (Larsell H VI; see Figure 4).

However, we did also observe a decrease of activation over time for the non-trained right hand movement during finger sequence performance in the contralateral cerebellar hemisphere (Larsell H VI and VIIA), as well as ipsilateral in SPL (BA 5) and ipsilateral cerebellar vermis (VI and VIII A).

For the right hand we found no significant results for post minus pre comparisons for writing and fist clenching.

**Correlation analysis with motor outcome measures (functional units):**

Correlation analysis of BOLD-magnitude changes during performance of the grip-force task showed no association with improvement in force over training for the trained and the non-trained hand side.

We found a negative association between the improvement for the visually guided task and activation magnitude in the left SPL (BA 7; t= 7.98; highest voxel in MNI-coordinates: -33, -48, 66) reaching into left M1 (BA 4; t= 7.98; highest voxel in MNI-coordinates: -30, -48, 66) and in the right cerebellar hemisphere crus 1 (t= 5.27; highest voxel in MNI-coordinates: 48, -48, -39) during writing with the left hand. The more the performance improved for left hand writing, the more the activity decreased in the dominant SPL/M1 and crus 1 during writing.

When we tested what behavioral change over training time (increase of the AAT tasks performance for the trained left hand) was associated with the initial fMRI-activation for the global performance increase, we found a predictive value for anterior cerebellar hemisphere activation in all fMRI-tasks. Whereas increase of tapping performance with the left hand was associated with right anterior cerebellar activation (t=6.56; MNI-coordinates: 18, -60, -27), increase of visually guided movements was associated with left cerebellar activation during
writing \((t=5.90; \text{MNI-coordinates: } -30, -57, -33)\) but also with left putamen activity \((t=5.55; \text{MNI-coordinates: } -24, 9, -9)\).

The motor transfer of performance for the right hand was not associated with relevant activation changes in the ROIs selected.

**Discussion**

Imaging studies investigating longer periods of motor training are rare, although training time in a range of weeks to months are those essential to understand for discussing effects observed in motor rehabilitation. We therefore applied a comprehensive long-term motor training for the non-dominant hand to investigate behavioral and imaging effects for the trained and the non-trained hand. We also intended to use a paradigm in healthy volunteers, which should be at least partially congruent to a training procedure important in patients after cerebral damage such as stroke. However, healthy young participants might not improve performance when applying this training procedure to the dominant hand side. In order to avoid a ceiling effect for healthy volunteers we trained the left non-dominant hand in strongly right handed individuals.

The selection of the motor task for fMRI is crucial for detecting changes in brain activation observed during training. In this study we intended to measure activation sites associated with movements of the trained and the non-trained hand and therefore scanned movements of each task with each hand independent from another and randomized the temporal order of measurements. In addition, we intended to keep pre- and post-performance balanced over time. Therefore we selected a fist clenching task balanced for effort (33% of maximal force for each hand [39]) but also a finger sequence task [40] unbalanced for effort but balanced for frequency of finger taps (1Hz). In order to also measure highly automatized movements with a strong recruitment of the same dominant hemispheric representations for both hand performance [1] we used a writing task with maximal velocity performance. For writing the velocity was adapted to the individual cognitive strain (no difference between pre- and post-measurement) and we were therefore able to measure a transfer effect of AAT on the motor performance in the fMRI-scanner \((\text{number of words written increased for the trained but not for the untrained hand})\). The fist movement and finger sequence task have been successfully applied in studies with stroke patients and results observed in a training study in healthy participants might be valuable to compare with training results in the patient group [34,41].

Two weeks of a specific motor training (AAT) of the left, non-dominant hand resulted in an increase of performance of the left trained hand over the training period. However, there were marked differences on the hand-transfer of training induced performance increase between conditions: Whereas visually guided movements showed a training effect especially for the trained hand performance, sequential finger movements showed a comparable training increase for both hands. Overall, we found training associated reductions in functional activation which were located in the dIPFC in areas associated with working memory [42], ipsilateral primary somatosensory, parietal, and bilateral supplementary motor cortex for the finger sequence task and in the ipsilateral posterior cerebellar hemisphere during writing. Reductions seen in ipsilateral posterior cerebellar hemisphere (crus 1) and the dIPFC fit well to the assumption that training is associated with decreased cognitive load. Whereas the dIPFC seemed to be especially involved in the transfer of numbers presented on button presses which is increasing working memory load, the posterior cerebellar hemisphere seems to process increased attention during writing using the non-dominant hand. In previous studies ipsilateral posterior cerebellar
activation in crus 1 has already been associated with increased attentional demand and cognitive function of the cerebellar hemisphere [10,43]. Our finding might therefore indicate that the performance of the task required less attention after the AAT.

As well as for the left hand performance, we found a training associated activity decrease for the right hand performance of sequential finger movements, too. However, the observed activity decrease during sequential finger movements took predominantly place in the cerebellum and in the superior parietal cortex. Studies on motor sequence learning have shown that the cerebellum is not essential for the performance of highly trained motor sequences [18,20,21]. Our finding of a decreased activity in the cerebellum after the AAT therefore might indicate economization processes even in the case of non-trained hand performance of sequential finger movements. This would be in accordance with the hypothesis of Seidler [44] that transfer of learning is on the neuronal level similar to the late phase of learning, even if this consideration is predominantly based on the literature of motor adaptation.

In contrast, anterior cerebellar hemisphere activation is associated with sensorimotor feedback loops maintaining a „forward model” of the motor apparatus, used to predict the sensory consequences of actions [11,20,45,46]. In our study anterior cerebellar activation increased over time for the writing task together with an increasing performance velocity for the left hand. An increased recruitment of trained anterior cerebellar loops might therefore be the neuronal substrate of increased writing performance. Initially high anterior cerebellar hemisphere activation predicted for a high training increase of both sequential and visually guided movements. The processing of sensorimotor interaction during motor performance might well be increasingly necessary for the writing in comparison to the finger sequence task, a finding which had also an impact on the reduction of sensorimotor representation during training. In addition, writing recruited the cerebellar vermis which might well be associated with rhythmic components of the writing movement [47]. In contrast, finger sequences were externally pulsed and in a very slow and rhythmically non-demanding velocity.

Overall, the changes for the representation of sequential finger movements observed in our study during training fit partially well with the model of Robertson et al. [12] suggesting that motor sequence learning is more associated with frontal cortical (DLPFC, SMA, dPMC) activation. Over time cortical bilateral activation during performance of finger sequences focus on primary motor cortex and dPMC of the hemisphere contralateral to the executing hand. This has been repeatedly described for sequential finger movements ([2]; [3]; [4]). Highly trained expert musicians in comparison to non-musicians, show decreased motor activations within the SMA, the PMC, and the ipsilateral M1 during movement performances of varying complexities ([2]; [5]). In addition, it has been demonstrated that this reduced activation is also present after transfer on new finger sequence tasks [48].

We found a considerable training transfer on the non-trained dominant hand. Intermmanual learning transfer has been previously described [24] and a high task-dependency of intermanual transfer has also been reported [25]. Whereas in our study visually guided movements showed a training effect especially for the trained hand performance, sequential finger movements showed a comparable training increase for both hands. In our right handed sample, writing predominantly recruits resources of the dominant hemisphere, even when performed with the left hand, a finding reported before [1]. The conjunction analysis (Figure 3) revealed that this is in contrast to the sequential finger tapping task for which common resources for both hand sides are represented without hemispheric dominance. In addition, predominantly the trained left hand profits from AAT training for the group of visually guided movements since there is a
high ceiling effect for the right dominant hand. In contrast, finger sequencing is less lateralized, there is less ceiling effect for the right hand and consequently, also the right hand is profiting from left hand training in these tasks. However, we found no relevant associations between right hand performance increase for sequential movements and increase in BOLD-magnitude.

We found an increase of activation only in the anterior cerebellar hemisphere ipsilateral to the executed writing movement, and the bilateral putamen during the fist clenching task. In particular, the basal ganglia and the cerebellum are related to instrumental, cognitive, and behavioral automatization that has been linked to expertise and giftedness. Several previous imaging studies investigating healthy subjects with extremely sophisticated motor expertise [21,49] found an economization of cortical representation sites and increased recruitment of the basal ganglia network with increasing levels of expertise. Overall, it seemed to be plausible to us to draw comparisons with respect to substantial considerations on activity changes in the corticostriatal and corticocerebellar systems relating to established motor learning paradigms [18,21], i.e. motor sequence learning [20] and motor adaptation [44].

Most interestingly in a metaanalysis Hardwick and colleagues [19] recently identified putamen activation as typical for sensorimotor guided motor learning. In our long-term training this change over time was especially evident for the visually feedback fist clenching task. This task was completely unusual at the beginning for all participants and might therefore be well suited for demonstrating automatization associated changes over training in the sensorimotor system.

We did not observe any increased activation in the primary sensorimotor cortex contralateral to the trained hand for our paradigm. This has described after short term repetitive training [50] but also for long-term training [51,52]. Karni and colleagues [51,52] observed a subsequent enlargement of cortical activity in the contralateral primary motor cortex (cM1) during learning (and repetition) of rapid finger movement sequences within the period of a few weeks (after an initial decrease) that persisted for 4 weeks to several months post-training. In contrast to these studies, we did not use the same paradigms for fMRI-mapping and training. For a transfer of paradigms an enlargement of M1 representation might be absent. Furthermore, an increase of representation area in TMS is only present within the first 30 minutes after repetitive training [53] an experimental situation also showing increased contralateral M1-representation with fMRI [50]. In the present fMRI was applied several hours after the last training session and short term activation and excitability increase might already be at baseline again.

Limitations

The high working load for long-term training limited the number of participants to fifteen. There might be more effects observed after training when the number of participants would be higher. In addition, effects over time and habituation in the scanner have not been controlled for. This would need a control group without being involved in the training. Furthermore, the analysis of “number of readable words” for the performance of the writing task during fMRI is unsatisfactory. A more detailed performance analysis for writing using kinematic analysis might be more appropriate.

Conclusions
Our study showed that two weeks of comprehensive training of the non-dominant hand results in healthy adults in a substantial effect for the trained (approx. 30%) but also for the untrained right dominant hand (approximately 19%). More specific performance increase for the left hand was demonstrated for visually guided movements (AAT tasks aiming, crossing circles and labyrinth). Moreover, our study identified reduced activation over training of cortical (ipsilateral primary sensorimotor, secondary motor areas and parietal cortex) and cerebellar (posterior inferior hemisphere) representation sites. In addition, movement automatization was seen in increased pallidum and putamen activation after training for the simple fist clenching movements. Correlation analyses revealed a relevant association between decrease of the dominant SPL/M1 and crus 1 activation and increase in writing performance. Overall, cerebellar but also basal ganglia structures were differently engaged during different time points of motor learning. During the pre-condition the cerebellar hemisphere crus 1 was increasingly active, indicating increased attentional and cognitive processes. During the post-measurement the anterior cerebellar hemisphere and the putamen showed increased activation, indicating increased sensorimotor feet forward processing and automatization. A high initial anterior cerebellar and putamen activation predicted for a better training effect.

Further studies might use the model developed here to specifically investigate effects of therapy modulations (transcranial cortical excitability modulation, somatosensory stimulation) prior to motor training and differences in changes induced by motor training with age or induced by pathology (stroke, traumatic brain injury).

**Acknowledgement and Grants**

This study was supported by the DFG Lo 795/7-1.

**Declaration**

Each author made an important contribution to the manuscript. The authors declare that they have no conflicts of interest.
References


Table 1: Conjunction over fMRI- tasks and time (FWE corrected over the whole brain)

<table>
<thead>
<tr>
<th>Region</th>
<th>BA/ Larsell</th>
<th>t-value</th>
<th>MNI-coordinate</th>
<th>t-value</th>
<th>MNI-coordinate</th>
<th>t-value</th>
<th>MNI-coordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
<td>x</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td>M1 le</td>
<td>4</td>
<td>5.25</td>
<td>-51</td>
<td>-24</td>
<td>42</td>
<td>8.53</td>
<td>-45</td>
</tr>
<tr>
<td>M1 ri</td>
<td>4</td>
<td>7.63</td>
<td>45</td>
<td>-30</td>
<td>45</td>
<td>6.85</td>
<td>-27</td>
</tr>
<tr>
<td>S1 le</td>
<td>1,2,3</td>
<td>10.95</td>
<td>-42</td>
<td>-36</td>
<td>45</td>
<td>6.92</td>
<td>-27</td>
</tr>
<tr>
<td>S1 ri</td>
<td>1,2,3</td>
<td>9.02</td>
<td>42</td>
<td>36</td>
<td>45</td>
<td>5.21</td>
<td>33</td>
</tr>
<tr>
<td>dPMC le</td>
<td>6</td>
<td>5.20</td>
<td>-36</td>
<td>-12</td>
<td>54</td>
<td>10.48</td>
<td>-27</td>
</tr>
<tr>
<td>dPMC ri</td>
<td>6</td>
<td>8.68</td>
<td>27</td>
<td>-6</td>
<td>51</td>
<td>6.11</td>
<td>27</td>
</tr>
<tr>
<td>SMA le</td>
<td>6</td>
<td>7.47</td>
<td>-3</td>
<td>-6</td>
<td>60</td>
<td>9.01</td>
<td>-6</td>
</tr>
<tr>
<td>SMA ri</td>
<td>6</td>
<td>6.17</td>
<td>9</td>
<td>-2</td>
<td>60</td>
<td>9.52</td>
<td>0</td>
</tr>
<tr>
<td>vPMC le</td>
<td>6,44</td>
<td>5.83</td>
<td>-57</td>
<td>6</td>
<td>27</td>
<td>7.02</td>
<td>-57</td>
</tr>
<tr>
<td>vPMC ri</td>
<td>6,44</td>
<td>6.16</td>
<td>54</td>
<td>3</td>
<td>39</td>
<td>6.06</td>
<td>57</td>
</tr>
<tr>
<td>SPL le</td>
<td>7</td>
<td>4.81</td>
<td>-36</td>
<td>-45</td>
<td>57</td>
<td>6.90</td>
<td>-35</td>
</tr>
<tr>
<td>SPL ri</td>
<td>7</td>
<td>5.09</td>
<td>-33</td>
<td>-42</td>
<td>54</td>
<td>6.15</td>
<td>30</td>
</tr>
<tr>
<td>IPL le</td>
<td>40</td>
<td>5.76</td>
<td>-51</td>
<td>-31</td>
<td>44</td>
<td>5.76</td>
<td>-51</td>
</tr>
<tr>
<td>IPL ri</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1-2 le</td>
<td>17,18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1-2 ri</td>
<td>17,18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V5 le</td>
<td>19</td>
<td>7.12</td>
<td>-42</td>
<td>-69</td>
<td>3</td>
<td>5.21</td>
<td>-42</td>
</tr>
<tr>
<td>V5 ri</td>
<td>19</td>
<td>5.21</td>
<td>45</td>
<td>-66</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB ri</td>
<td>L VI</td>
<td>5.37</td>
<td>27</td>
<td>-64</td>
<td>-19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: fMRI - comparisons pre versus post

1 Brodmann’s area (BA); Larsell: Larsell’s lobule
2 M1: primary motor cortex; ri: right, le: left
3 S1: primary motor cortex
4 dPMC: premotor cortex, d: dorsal; v: ventral
5 SMA: supplementary motor area
6 SPL: superior parietal lobe
7 IPL: inferior parietal lobe
8 V1-2: primary visual cortex
9 V5: visual motion area
10 CB: cerebellar hemisphere
<table>
<thead>
<tr>
<th>Region</th>
<th>BA/Larsell</th>
<th>t-value</th>
<th>MNI-coordinates</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fist clenching 1Hz left; post minus pre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pallidum right</td>
<td>3.53</td>
<td>24</td>
<td>-6</td>
<td>-3</td>
</tr>
<tr>
<td>putamen right</td>
<td>3.59</td>
<td>27</td>
<td>-6</td>
<td>-6</td>
</tr>
<tr>
<td>putamen left</td>
<td>4.10</td>
<td>-18</td>
<td>9</td>
<td>-6</td>
</tr>
<tr>
<td>Finger sequence 1Hz left; pre minus post</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dorsolateral PFC</td>
<td>8</td>
<td>5.08</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>i-S1 13 left</td>
<td>1,2,3</td>
<td>4.14</td>
<td>-42</td>
<td>-39</td>
</tr>
<tr>
<td>i-SMA 14 left</td>
<td>6</td>
<td>3.99</td>
<td>-9</td>
<td>15</td>
</tr>
<tr>
<td>c-SMA right</td>
<td>6</td>
<td>3.82</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>i-SPL 15 left</td>
<td>5</td>
<td>4.59</td>
<td>-12</td>
<td>-51</td>
</tr>
<tr>
<td>i-SPL left</td>
<td>7</td>
<td>4.32</td>
<td>-9</td>
<td>-54</td>
</tr>
<tr>
<td>i-IPS 16 left</td>
<td>40</td>
<td>3.90</td>
<td>-39</td>
<td>-42</td>
</tr>
<tr>
<td>cerebellum Crus 1 left</td>
<td>LVIIA</td>
<td>3.60</td>
<td>-42</td>
<td>-57</td>
</tr>
<tr>
<td>Finger sequence 1Hz right; pre minus post</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i-SPL left</td>
<td>5</td>
<td>4.03</td>
<td>6</td>
<td>-57</td>
</tr>
<tr>
<td>cerebellar hemisphere left</td>
<td>L VI</td>
<td>4.69</td>
<td>-12</td>
<td>-75</td>
</tr>
<tr>
<td>cerebellar Crus 1 left</td>
<td>LVIIA</td>
<td>4.55</td>
<td>-12</td>
<td>-75</td>
</tr>
<tr>
<td>cerebellar vermis</td>
<td>L VIII</td>
<td>3.81</td>
<td>3</td>
<td>-63</td>
</tr>
<tr>
<td>cerebellar vermis</td>
<td>L VI</td>
<td>3.57</td>
<td>3</td>
<td>-69</td>
</tr>
<tr>
<td>writing left; pre minus post</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cerebellar Crus 1 left</td>
<td>LVIIA</td>
<td>4.34</td>
<td>-30</td>
<td>-78</td>
</tr>
<tr>
<td>writing left; post minus pre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cerebellar hemisphere left</td>
<td>L VI</td>
<td>4.54</td>
<td>-33</td>
<td>-69</td>
</tr>
</tbody>
</table>

**Figures**

11 Brodmann’s area (BA)
12 p-values were FWE corrected
13 i-S1: ipsilateral primary somatosensory cortex
14 c-SMA: contralateral supplementary motor area
15 SPL: superior parietal lobe
16 IPS: intraparietal sulcus
* corrected over the whole brain volume with FWE
** with FDR
Figure 1:

Percentual improvement for the each of the tasks of Arm Ability Training (AAT) over the whole intervention for the left trained and the right non-trained hand.

Figure 2:

Top: Illustration of training procedures.

Middle: Illustration of improvement of the left hand during two weeks of training provided in the averaged time (over sessions and subjects) needed for task performance (milliseconds). In contrast to the grip force unit subjects improved continuously for the sequential finger and visually guided movements.
Bottom: Percentual improvement and its standard deviation of the trained left and non-trained right hand performance for A) sequential finger movements and B) visually guided movements C) grip force. Grey bars demonstrate left hand, black bars right hand improvements. Standard deviations are plotted above bars; both hands improved comparably for the sequential finger movements (non-significant (n.s.) differences between their improvements). On the contrary, the trained left hand showed a highly significant (p<.001, indicated with ***) increased improvement for the visually guided movements.

Figure 3:
Conjunction analysis for the fist clenching, the sequential finger movements and the writing task over both hands and both time units. For the fist clenching task left M1, dPMC and vPMC, right SMA, bilateral SPL, left IPL, bilateral V5 and right anterior cerebellar hemisphere showed relevant activation. Sequential finger movements showed a bilaterally symmetric activation pattern in the SMA, dPMC, vPMC (reaching into BA 44), M1, S1, SPL and occipital lobes. The lateral cerebellar hemisphere was only activated on the right side. In contrast, writing showed an activation pattern for the same areas lateralized to the dominant cerebral hemisphere. Only BA 44, vPMC and SPL and cerebellar posterior hemisphere were activated on the right side. The superior vermis was also contributing to writing of both hand sides.
Figure 4:

Training-associated decrease of activations in regions of interest during left finger sequence performance.

Left: Fist clenching: no effect for the pre minus post comparison. Increased activation after training was found in the bilateral pallidum and putamen (post minus pre).

Middle: Finger sequence: The pre minus post comparison revealed activation differences in the superior parietal lobe (SPL, BA 5; x=42), dorsolateral prefrontal cortex (DLPFC; x=33) and bilateral supplementary area (SMA; z=63). No significant effect was found for the post minus pre comparison.

Right: Writing: The pre minus post comparison showed an effect in the ipsilateral posterior-inferior cerebellar hemisphere (x=-30; z=-36). The post minus pre comparison showed an effect in the ipsilateral anterior cerebellar hemisphere (x=-33; z=-21).
Figure 5:

Results of the correlation analysis.

A: The more the performance improved for left hand writing during training, the more the activity decreased in the dominant SPL (middle; $t=7.98$; highest voxel in MNI-coordinates: -33, -48, 66) and crus 1 (bottom; $t=5.27$; highest voxel in MNI-coordinates: 48, -48, -39) during writing.

B: High left cerebellar activation during writing ($t=5.90$; MNI-coordinates: -30, -57, -33) but also left putamen activity ($t=5.55$; MNI-coordinates: -24, 9, -9) before training predicted high performance gain for visually guided movements during training.

C: Right anterior cerebellar activation ($t=6.56$; MNI-coordinates: 18, -60, -27) before training during the finger sequence task predicted high performance gain in left side finger tapping over training.
Supplementary Files:

Supplementary Figure 1A: Main effect of finger sequence and writing for the left hand pre and post
Supplementary Figure 1B: Main effect of finger sequence and writing for the right hand pre and post