Aus der Klinik für Psychiatrie und Psychotherapie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin Charité
Campus Mitte

DISSERTATION

Structural changes in motor tracts of chronic stroke patients associated with experimental rehabilitation: a DTI study

zur Erlangung des akademischen Grades

Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät

Charité – Universitätsmedizin Berlin

von

Felix Betzler

aus Sao Leopoldo/Brasilien

Gutachter/in:

1. Prof. Dr. med. F. Bermpohl
2. Prof. Dr. med. G. Juckel
3. Prof. Dr. med. E. Calzia

Datum der Promotion: 3. Juni 2012

Danksagung

Ich bedanke mich sehr herzlich bei meinem Doktorvater Herrn Prof. Felix Bermpohl für die umfassende Betreuung der Arbeit.

Weiterhin bedanke ich mich bei Herrn Prof. Gottfried Schlaug für die Betreuung während der Datenerhebung am BIDMC - Harvard Medical School, sowie den Mitarbeitern seiner Arbeitsgruppe für die Unterstützung bei der Arbeit.

Meiner Familie Danke ich für die Anteilnahme und die Unterstützung, die mir die Arbeit ermöglichte.

Großer Dank für Unterstützung gilt weiterhin Charles Li Hui, Ludwig Schlemm, Stephan Köhler und Janina Stiefel.

Contents

1. Figures

2. Tables

3. Equations

4. Abbreviations

ADC	Apparent diffusion coefficient
AI	Asymmetry index
aMF	Alternate motor fibers
CRT	Corticorubrospinal tract
CV	Coefficient of variation
DTI	Diffusion tensor imaging
EEG	Electroencephalography
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
ICC	Intraclass coefficient
MRI	Magnetic resonance imaging
PT	Pyramidal tract
ROI	Region of interest
tDCS	Transcranial direct current stimulation
UE-FM	Upper extremity Fugl-Meyer score
RF	Reticular formation
WD	Wallerian degeneration
SD	Standard deviation
PLIC	Posterior limb of the internal capsule

5. Introduction

5.1 Overview of the study's aims

This chapter will briefly outline the principal aim of the current study. A detailed description of the study's purpose will follow (chapter 7) after the theoretical background (anatomy and relevant imaging-/stimulation-techniques) has been described (chapter 5.2 - 5.4).

The principal aim of the study is to investigate potential neuroplastic changes in stroke patients as a response to an intense experimental rehabilitation program. The program consists of combined brain stimulation (transcranial direct current stimulation) and motor training. With the use of Diffusion Tensor Imaging, potential structural changes will be investigated longitudinally in a pre-post-treatment comparison.

In order to address this question, two methodical aspects need to be established:

First: the design of a reproducible protocol for fiber-tracking of certain tracts of interest using Diffusion Tensor Imaging. The protocol targets two tracts of the motor system: the pyramidal tract and a group of several motor pathways which we consolidated as "alternate motor fibers" (see chapter 5.2).

Second: evaluation of the reproducibility of the protocol and assessment of the variability in healthy subjects within the following categories: variability between subjects *(inter-subject variability)*, between scans, i.e. comparing the scans of two different timepoints *(inter-session variability)*, and between sequences: within one scan-session, several scans ("sequences") have been collected *(intra-session variability)*

The protocol, along with an assessment of motor skills, is then applied to the group of chronic stroke patients before and after receiving the rehabilitation treatment. Potential neuroplastic changes during the period of recovery which are thought to be reflected by certain changes in diffusivity (see chapter 5.3) are investigated.

5.2 Anatomical and functional background of the motor system

The aim of this section is to give an overview of the anatomy and function of the motor system. It focuses on the parts of the motor system that have been investigated in the present study, i.e. the primary motor cortex, the corticospinal tract also known as pyramidal tract (PT), the corticorubrospinal tract, the corticoreticulospinal tract and descending autonomic fibers.

We separated the tracts mentioned above into two parts. The pyramidal tract represents one part, whereas all the other descendant corticospinal motoric pathways – such as the corticorubrospinal tract, the corticoreticulospinal tract and descendant autonomic fibers – form the second part. We will refer to this latter part as alternate motor fibers (aMF) hereafter [1]. The reason for separating the pyramidal tract from the alternate motor fibers is because the pyramidal tract can easily be distinguished from all other tracts both functionally and topographically [2]. Topographically, it is distinguishable by Diffusion Tensor Imaging (see chapter 5.3) since the pyramidal tract has a well described trajectory, passing through certain “landmarks” in the brain which makes it easily definable. Using Diffusion Tensor Imaging, other corticofugal motor fibers can only be evaluated as a consortium, since the technique is not capable of distinguishing small fiber bundles or single neurons from each other which have a similar trajectory. Figure 1 shows a representative image of the PT and aMF visualized by diffusion tensor imaging.

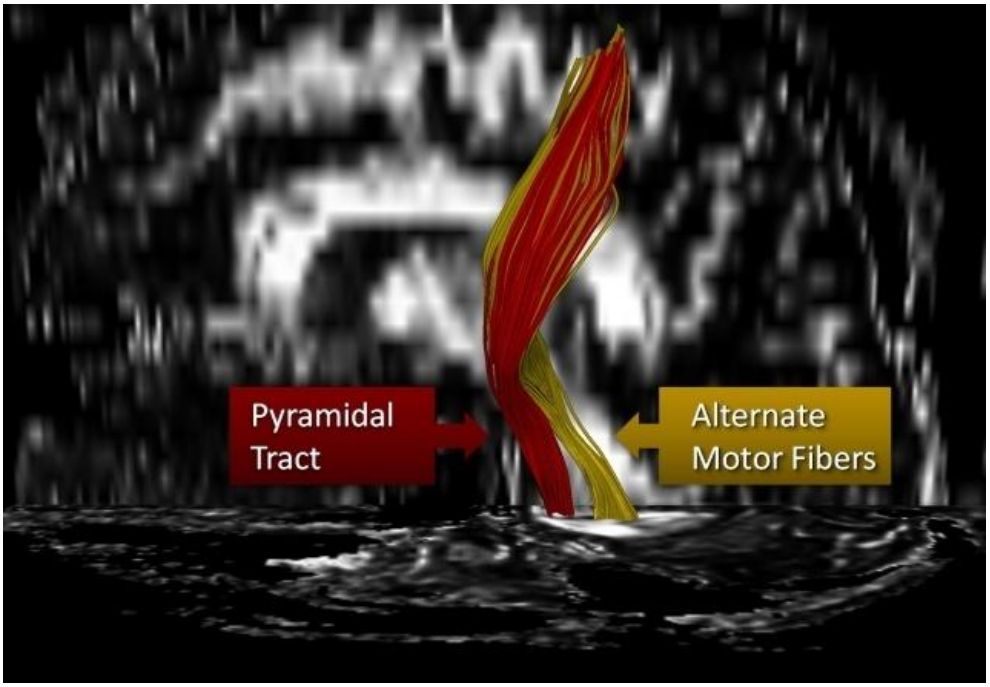


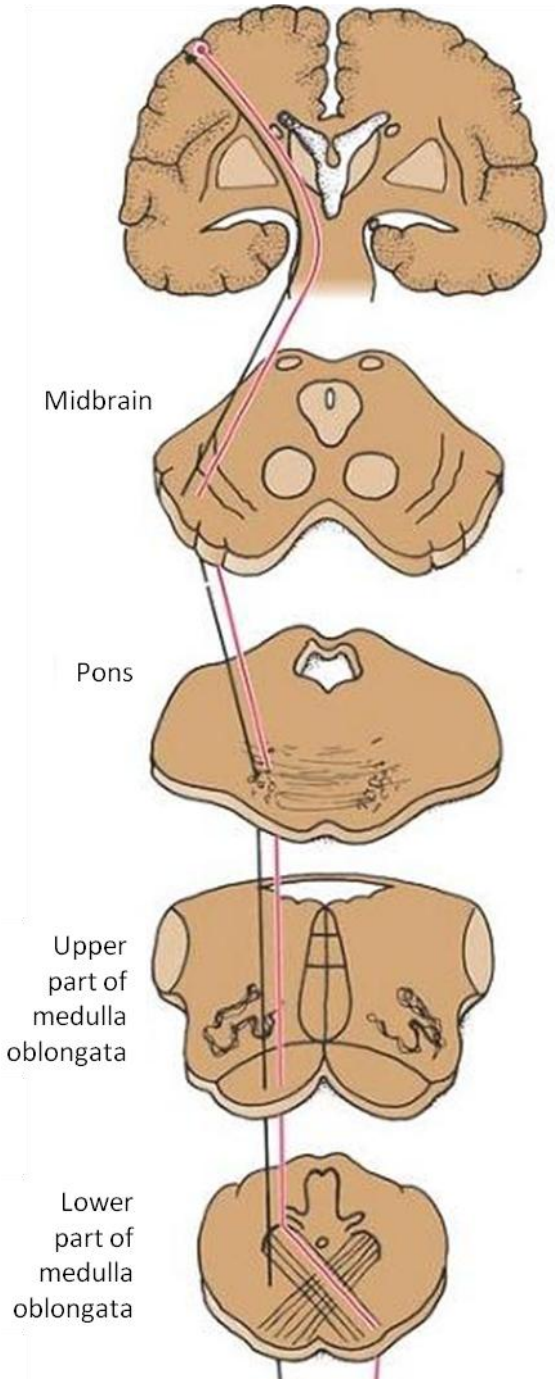
Figure 1: ***Motor tract reconstruction.*** *A lateral view of a reconstruction example of the pyramidal tract (red) and alternate motor fibers (yellow) of one of the healthy controls. Both tracts show a trajectory reaching from the primary motor cortex to the spinal cord. The alternate motor fibers separate at the level of the cerebral peduncles and take a pathway through the posterior portion of the pons.*

The pyramidal tract

The pyramidal tract arises from three different motor cortices: one-third stems from the primary motor cortex, one-third from the secondary motor cortex and one-third from the parietal lobe. The corticospinal fibers pass through the corona radiata and the posterior portion of the internal capsule. The PT traverses the midbrain through the basis pedunculi. Reaching the pons, the PT then fans out in several small fiber bundles as it is crossed by the transverse pontocerebellar fibers. These bundles converge again in the anterior medulla oblongata, forming a prominent structure, the so called pyramids.

Approximately 75% of the pyramidal fibers cross in the pyramidal decussation situated at the junction of the medulla and the spinal cord, while 25% remain uncrossed [3]. Of those uncrossed fibers, 15% decussate further caudally in the spinal cord innervating

the contralateral side. Another part (10%) remains uncrossed [4, 5]. Figure 2 shows the pathway of the PT through the different portions of the brain.



Alternate motor fibers

Corticorubrospinal tract

The corticorubrospinal tract (CRT) reaches from the cortex to the spinal cord. It has a synaptic interstation in the form of the red nucleus. The red nucleus receives afferent fibers from the ipsilateral cortex (areas 4 and 6) which are synaptically connected to neurons descending to the spinal cord. It also receives afferent impulses from the cerebellum [6]. The red nucleus is located in the tegmentum of the midbrain. From here, after crossing the midline, the fibers descend through the pons and the medulla oblongata to enter the spinal cord (lateral white column).

Figure 2: ***Pathway of the pyramidal tract.*** *Slices are depicted from a frontal view on the brainstem. Adapted from Snell, Clinical Neuroanatomy, 2010 [7].*

Corticoreticulospinal tract

The reticular formation (RF) is a group of many neural networks and is located throughout the midbrain, pons and medulla oblongata. The RF, particularly the pontine and medullar nuclei of the RF on both sides, receive afferent fibers from area 6 which descend along with the fibers of the PT. From here two separate fiber bundles descend to the spinal cord: the medial and the lateral reticulospinal tract which remain mostly uncrossed and are not somatotopically organized. The corticoreticulospinal pathway is also thought to include the descending autonomic fibers. These fibers are not one distinct tract but rather separate corticospinal fiber bundles arising from the higher centers of the central nervous system located in the cerebral cortex, hypothalamus, amygdaloid complex, and reticular formation [7].

5.3 Diffusion Tensor Imaging (DTI)

5.3.1 Basis of molecular diffusion

The present study employs Diffusion Tensor Imaging (DTI), which is an MRI technique providing information about diffusion of water molecules in the brain. If one assumes that this diffusion happens without any anatomical constraints, water molecules would randomly diffuse in all spatial directions and the diffusion pattern could be visualized as a three dimensional sphere which describes potential locations of the water molecule. This way of diffusion is commonly referred to as isotropy or isotropic diffusion [8, 9]. If water molecules are constrained in diffusion, e.g. by axonal membranes or myelin sheaths, the diffusion behavior of water molecules changes. Dependent on the barrier, water molecules are now able only to diffuse in certain directions. Consequently, overall diffusivity decreases and diffusion becomes directional, according to the anatomical constraints. This behavior of water molecules is known as anisotropy or anisotropic diffusion and the shape of the molecules' potential locations can now be described as an ellipsoid rather than a sphere (see figure 3).

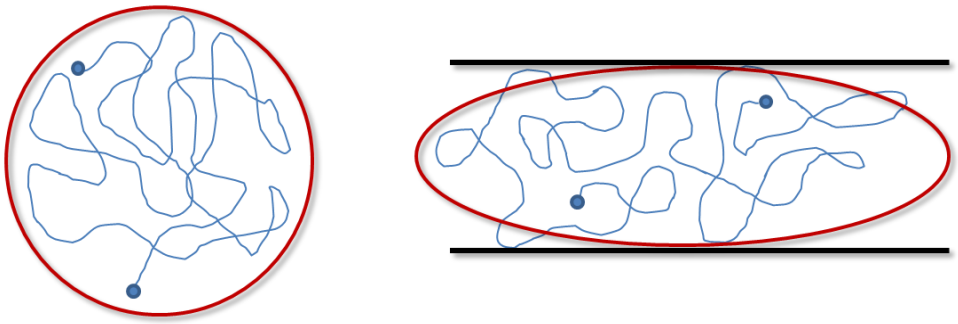


Figure 3: ***Unrestricted/restricted diffusion patterns.*** *The model on the left describes unrestricted diffusion; the sum of potential locations of the water molecule can be described as a sphere whereas in restricted diffusion it becomes ellipsoid (right).*

In order to depict anisotropic diffusion in a three dimensional space, the calculation of a diffusion tensor is needed. The tensor consists of three different eigenvectors and three eigenvalues accordingly. Eigenvectors are assigned to v (v_1, v_2, v_3) and represent the directions of the principal axes that define the ellipsoid while the eigenvalues are expressed by λ (λ_1, λ_2, λ_3) and represent the length of the vectors. The tensor provides information about the direction of diffusion: the main diffusion has the direction of the eigenvector with the highest eigenvalue (λ_1) allowing conclusions to be drawn about the probability of a fiber trajectory (see figure 4).

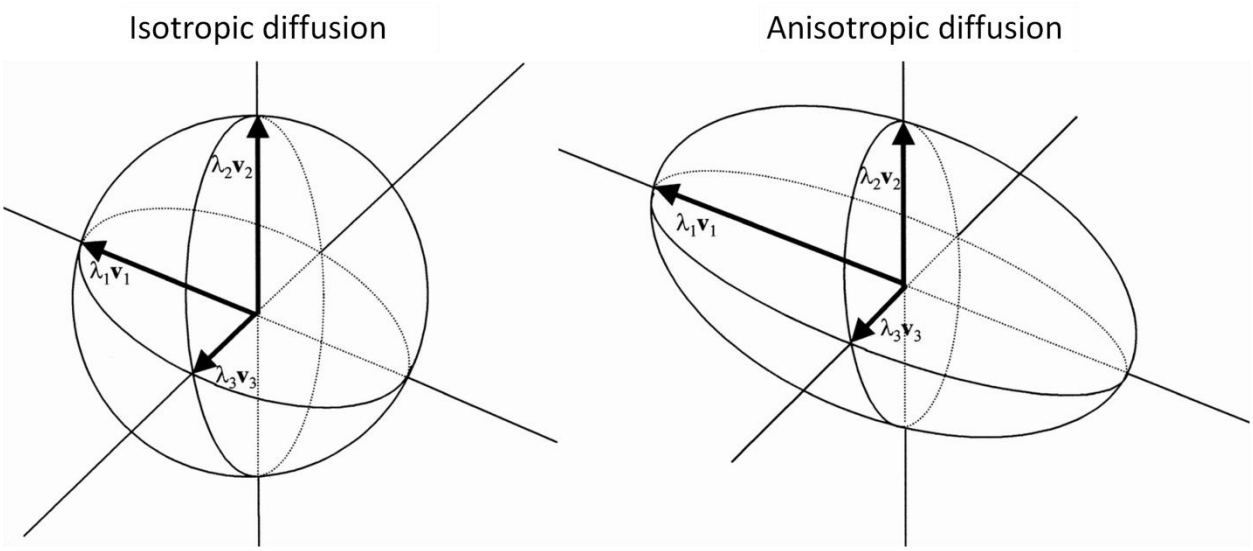


Figure 4: ***Three-dimensional model for isotropic and anisotropic diffusion.*** *The figure shows an example of isotropic diffusion (left) where all eigenvalues have about the same magnitude ($\lambda_1 \sim \lambda_2 \sim \lambda_3$). On the right, it shows an example of anisotropic diffusion where the principal eigenvector v_1 with the eigenvalue λ_1 determines the orientation of the ellipsoid since $\lambda_1 > \lambda_2$ and $\lambda_1 > \lambda_3$. Adapted from Wiegell et al., 2000 [10].*

Based on the three eigenvalues, a normalized scalar rotation-invariant measure can be calculated: fractional anisotropy (FA). FA is expressed as a value between 0 (when diffusion is isotropic) and 1 (when diffusion is constrained along one axis only). Equation 1 describes the calculation of FA

$$\mathrm{FA} = \sqrt{\frac{1}{2}}\frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

Equation 1: ***Equation for the calculation of fractional anisotropy (FA).*** *FA is calculated based on the eigenvalues (λ_1, λ_2, λ_3); FA-value of 0 = isotropic diffusion; FA-value of 1 = anisotropic diffusion*

The type of constraints can be manifold: diffusion of water molecules can be modulated by cellular membranes, neurofilaments, microtubules, myelin sheaths, glial cells or physiological processes. However, anatomical constraints are thought to weigh more than physiological processes [11]. Anatomical constraints in turn can be divided again by their impact of altering diffusion in the brain. Studies have shown that axonal membranes are the major factor influencing diffusion of water molecules, while the degree of myelination seems to play a minor role and only modulates anisotropic diffusion [11, 12].

5.3.2 Visualization of DTI

DTI images can be visualized in two or three dimensions. In the 2D view, the image can be coded by several parameters, e.g. FA or axis-designated colors. The color-coded map depicts the angle of the ellipsoid's main vector by assigning a color to the dominant angle of each voxel: angles along or close to x-axis=red, to y-axis=green, to z-axis=blue. Every possible angle can be coded by depicting each voxel as a mixture of the above-mentioned colors. They become purer the more they match the corresponding axis. FA maps are generated by assigning a gray scale value to each voxel according to its degree of fractional anisotropy and are commonly used if a particular region in a particular slice is of interest (see figure 5 for examples of color-coded and FA diffusion maps).

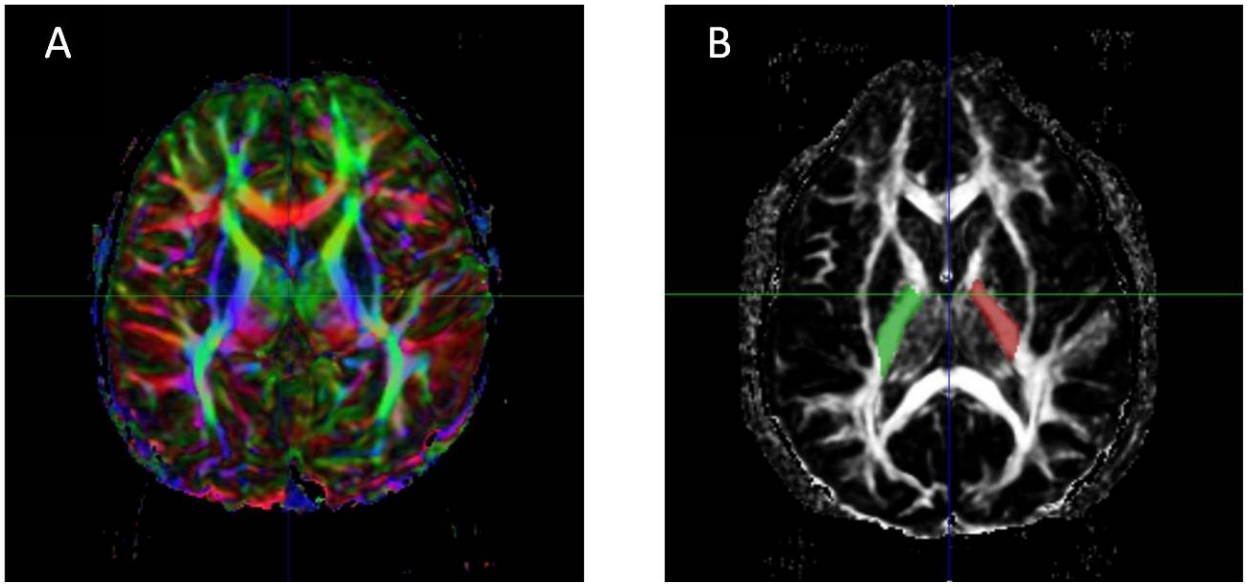


Figure 5: ***Color-coded/FA-coded axial slices of the brain.*** ***A:*** *color-coded map of diffusion. Each color is designated to a certain direction of diffusion: x-axis (left/right)=red, y-axis (frontal/dorsal)=green, z-axis (cranial/caudal)=blue.* ***B:*** *FA-map of a brain. The green and the red area in B represent regions of interest (ROI) used in this study, designating the posterior limb of the internal capsule.*

The second way of visualization is three-dimensional tractography. Using this technique, fiber tracts can be calculated by connecting adjacent voxels with similar principal eigenvectors. Based on a region of interest, tracts can be reconstructed, following the different paths of voxels with similar principal eigenvectors, assuming that they reflect the probable trajectories of the actual fiber tracts. However, this technique can only serve as a quantitative measure of connectivity between two different brain areas, not as an accurate correlate to the biological constitution of the brain. The calculation depends on manually definable parameters such as the FA-threshold or the angulation; adjacent voxels below the threshold or voxels which differ in their principal eigenvalues to a certain degree will not be taken into account.

In order to reconstruct a fiber which traverses location A, location B and location C, it is necessary to mark A, B and C as a region of interest (ROI) and to apply a logical AND-Function, which means that all fibers which traverse the ROIs are selected and visualized. As a result, a tract between A, B and C has been reconstructed, but it might

still be the case that the fiber-trajectory of certain fibers goes through all selected ROIs but also takes a pathway through other regions of the brain (location D). Hence, these fibers are not part of the targeted tract but still match the criteria which have been set so far. In order to exclude these fibers, another logical function is needed: the NOT-function. Applying the NOT-function in this case will enable the reconstruction of a tract with the logical function [[A and B and C] not D], which means all of the visualized fibers have to go through A, B and C but must not traverse D in any part of the trajectory. A graphical description of the AND-/NOT-function is shown in figure 6.

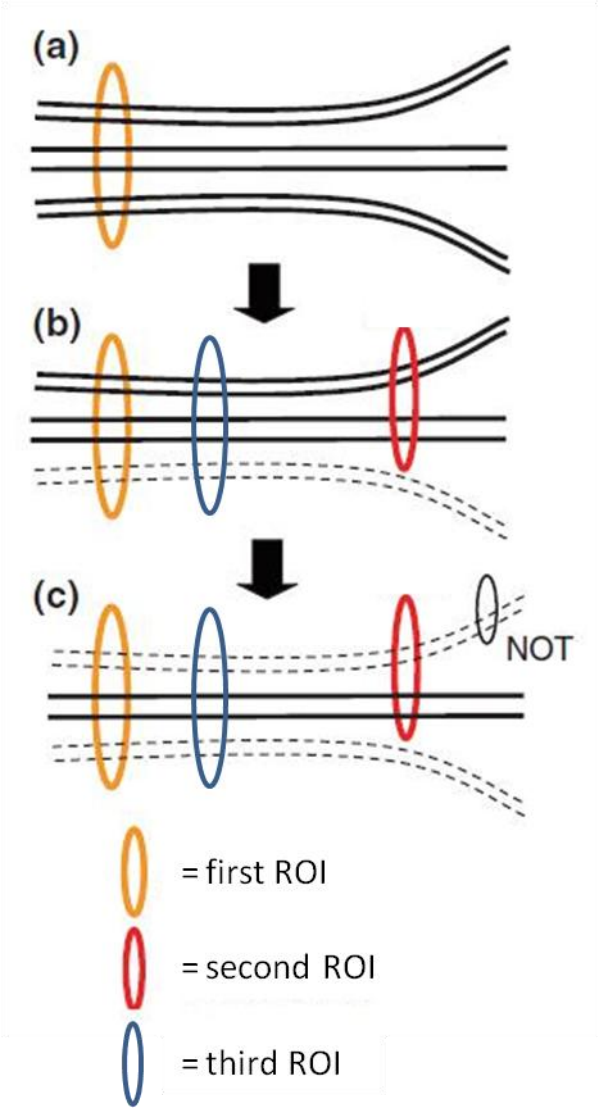


Figure 6: ***AND-/NOT-functions in tract reconstruction.*** *This figure shows an example of a tract reconstruction using the AND- and the NOT-function.* ***(a)*** *shows the visualized fibers after applying the first region of interest (ROI): all fibers traversing this region are retrieved.* ***(b)*** *after applying the second and third ROI with an AND-function, the lower part of the bundle is excluded, showing only the fibers traversing the first, second and third ROI.* ***(c)*** *a particular part which shall not be visualized may be excluded by adding a NOT-function. Adapted from Wakana et al., 2007 [13].*

5.4 Transcranial direct current stimulation (tDCS)

tDCS is a tool for modulating neuronal excitability which can be used for experimental and/or therapeutic purposes. In our study, we used tDCS for an experimental rehabilitation program after stroke. tDCS uses weak constant direct currents that are applied through the scalp. Electrodes soaked in isotonic sodium chloride solution (either anodal or cathodal) are placed over a targeted cortical area, and another electrode used as reference electrode is placed on the forehead. The stimulation device, a portable battery driven device, produces a direct current which traverses the skull inducing changes in the neurons' membrane potential. tDCS does not directly cause neurons to fire, but it modulates the excitability of the underlying brain tissue. This effect outlasts the time of stimulation. Nonsynaptic effects such as changes in ion fluctuations resulting in altered cellular ion concentration or conductance is one mechanism contributing to the effect of tDCS [14, 15]. Besides the nonsynaptic effects, synaptic changes are induced by the stimulation as well. The mechanism of synaptic connectivity is thought to play the major role in the tDCS effect and it is especially mediated by NMDA-receptors [16, 17]. Set-screws in tDCS stimulation are polarity and current-strength: anodal stimulation has been shown to up-regulate cortical excitability while cathodal stimulation has been shown to down-regulate cortical excitability due to processes of neural hyperpolarization and depolarization [18-20]. The current strength varies between the different studies while it generally lies in the range of <1-1.5 mA [21]. These currents can be considered reconcilable, as it has been shown that a current strength up to 25 mA/cm^2 does not cause damage to the brain tissue [22]. Parts of the current are being lost by the shunting through the scalp; however, the remaining currents reaching the underlying cortex are still sufficient to modulate excitability [23]. The effects of tDCS are not only dependent on polarity and current strength, but also on other factors. Gluckmann and Purpura suggested that the orientation of the dendritic-somatic axis relative to the electric field influences the effect of the stimulation [24, 25]. This means that little technical changes in the experiment setup may result in major changes in the clinical outcome. Additionally, the effect of stimulation is dependent on whether the stimulated area itself is excitatory or inhibitory and also on the baseline of activity of the targeted area and its afferent synaptic inputs [26].

6. Overview of current literature

This section will give an introduction to the current state of literature concerning DTI, tDCS and motor performance in stroke rehabilitation. It will also describe the gaps and questions that have not been addressed so far.
Previous studies of the following fields provide the basis for outlining the purpose of this study: DTI tract reconstruction in stroke patients, relation between tract integrity and motoric abilities in stroke patients, variability of DTI tract reconstruction.

The assessment of the corticospinal tracts in stroke patients with DTI in relation to their clinical outcome was investigated in several studies. Reports in recent years have related topographic proximity of stroke lesion to clinical scores [27-31]. A recent study investigated the quantitative relationship between the extent of structural damage to the CST and functional reorganization in chronic stroke patients. It found a positive, linear correlation between tract integrity and motor task-related cortical activity in stroke patients both lesionally and contralesionally in the primary sensorimotor cortex ventral to the hand area suggesting strong functional reorganization in both hemispheres [2].
Lindenberg et al., (2010) demonstrated negative correlations between motor impairment and DTI-derived measures of motor tract integrity: Asymmetry between hemispheres (due to low FA-values and small numbers of fibers) significantly predicted motor impairment. Both pyramidal tract and alternate motor fibers were investigated. Predictions were stronger when all motor tracts were combined as compared to predictions using only the pyramidal tract, suggesting that alternate motor fibers may play an important role in stroke recovery [1].
Several studies investigating the corticospinal tracts longitudinally after stroke provide insights about how stroke-related damage is associated with altered DTI-measures: along with the interruption of fiber tracts as it happens in stroke, wallerian degeneration (WD) causes changes in diffusivity in a distinct way; its effects have been investigated with DTI and its derived measures of ADC and FA [32-36]. Yu et al., conducted a longitudinal study, assessing diffusivity changes of the acute phase after stroke until one year post stroke. This study reported changes in diffusivity within the first weeks after stroke and a stabilization of all parameters of diffusivity after 3 months: FA-values decreased monotonously during the first 3 months and then stabilized. λ_1 decreased

during the first 2 weeks and then remained unchanged. $\lambda_{2,3}$ increased during the first 3 months and then stabilized. ADC increased after 2 weeks and stabilized after the third month [36].

DTI studies investigating the variability of fiber-tracking of the PT itself have shown that the different DTI-derived measures (fiber number, tract volume, FA value, ADC) varied in their reproducibility with fiber number showing the highest variability, followed by the tract volume. FA and ADC showed the lowest variability [37, 38]. It has also been shown that variability between different subjects (inter-subject variability) is higher than variability between two scans of the same subject at different timepoints (inter-session variability) [37].

The question whether potential neuroplastic changes can be assessed by DTI has not been applied to stroke patients that are in a chronic phase but undergo an experimental neurorehabilitation treatment; it has not been investigated whether a behavioral improvement as a result of rehabilitation therapy is associated with shifts in diffusivity in the motor system of the brain. Changes would provide experimental evidence for structural plasticity after neurorehabilitation and for the capability of DTI to assess this neural plasticity.

Several studies investigated the variability in DTI-derived measures in normal subjects but no study has yet related the variability to neuroplastic changes in chronic stroke patients: are DTI-derived measures precise enough to be able to capture neuroplastic changes?

Furthermore, assessments of the variability of DTI-derived measures in healthy subjects are still fragmentary: studies investigated both variability between subjects and variability between scans over two timepoints [37], whereas the variability between sequences (sequences = scans collected within one scan session) has not been reported so far.

7. Purpose of the study

This chapter will present the study's principal aims, followed by a detailed description of the approach we chose and the corresponding hypotheses we made.

Aims

The principal aim of the study is the investigation of potential neuroplastic changes in stroke patients as a response to an intense experimental rehabilitation program.

This general question is related to the following specific goals. A longitudinal pre-post-treatment comparison will address the question whether DTI-derived measures were capable to capture the structural impact of an intense rehabilitation program. The goal is to investigate whether the motor improvement - as a result to the rehabilitation program - is paralleled by a shift in a diffusivity pattern which may reflect neuroplastic changes.

In order to prove whether DTI-derived measures show longitudinal changes throughout the period of treatment that are suggestive of structural plasticity, two methodical aspects need to be established:

First: design of a robust protocol.
The protocol shall be able to assess the motoric system of the brain, in particular the pyramidal tract and alternate motor fibers. The reason we chose to investigate changes in descending corticospinal fibers is because first, our intervention targeted the motor impairment of our patients (accordingly, the stroke lesions affected the motor system) and second, because the anatomy of the motor system is well described and its visualization with DTI has been widely used in previous studies [27-31, 37-41].

Second: Evaluation of the protocol's reproducibility and assessment of variability.
The protocol shall be robust to the use of independent raters. Furthermore, applying the protocol to healthy subjects, variability will be assessed between subjects (inter-subject variability), between scans (inter-session variability) and between sequences within one session (intra-session variability).

Approach

The following paragraph will outline the steps of the design of the study in a chronological way:

1) Establishing a DTI fiber-tracking protocol which is capable of assessing the designated motoric corticofugal fibers (PT and aMF) of the brain and thoroughly testing this protocol concerning its reproducibility and reliability in healthy subjects. This includes various assessments: first, it requires an evaluation of the intra-rater reliability (test-retest reliability of the same rater) as well as the inter-rater reliability (agreement of two different raters) to assure the use of the protocol by independent investigators.
2) Quantifying the variability. The protocol requires three different assessments of variability as mentioned in the section above (“Aims”).
   First: inter-subject variability. In order to test whether the protocol is capable of reliably capturing our target tracts despite anatomical differences between individuals, we need to investigate the variability between subjects.
   Second: inter-session variability. This measure will test the variability between DTI-measures of one subject over two time points.
   Third: intra-session variability. This involves the comparison of two scan sequences of one subject which are collected within one scan session. This step provides information about the impact of influencing factors since we would expect physiological changes of microstructure or other factors to be much smaller within the duration of one scan session than between scans of two different timepoints.
3) Scanning stroke patients who are in a chronic phase and assessing both their diffusion pattern according to the protocol described above and their motor performance as a baseline.
4) Treating the patients with an intense rehabilitation program of tDCS and behavioral therapy.
5) Scanning the patients for the second time, assessing both the diffusion pattern and the motor outcome.

6) Comparing the patient group and the healthy age matched controls at baseline. Additionally, performing a longitudinal pre-post-treatment comparison in the group of stroke patients to assess potential neuroplastic changes. Finally, in order to address the question of whether the potential structural changes in patients exceed normal variability, the magnitude of change between pre-post-treatment evaluation to inter-session variability of the control group will be compared.

Hypotheses

Our general hypothesis is that stroke patients undergoing our rehabilitation program show a motor improvement which is paralleled by a shift in the diffusivity pattern in the motoric tracts.
Our specific hypotheses (related to the 6 steps of the section 'Approach') are:

Regarding 1) We expected the reproducibility of the protocol to be higher when performed by one rater than by two independent raters.
Regarding 2) We hypothesized a higher variability between subjects (inter-subject variability) relative to the variability between scans of one subject (inter-session variability) and also higher than variability between sequences within one session (intra-session-variability). Basis for this hypothesis is the assumption that anatomical differences between the subjects' brains will result in various different diffusion properties. We also hypothesized a higher inter-session variability relative to intra-session variability. The reason for this assumption is the possibility that intersession-variability may be influenced by factors which don't apply to intra-session variability such as physiologic changes in the brain within two timepoints due to clinical unapparent microstrokes, intensive training of certain brain regions, major position changes regarding scans 1 and 2 etc.
Regarding 3) - 5) We hypothesized that the patients would show a behavioral motor improvement as a response to our intervention.
Regarding 6) We expected the motor improvement in the patient group to be paralleled by changes in diffusivity in the motor system. Also, we expected these changes to exceed normal scan-to-scan variability of controls.

8. Materials and methods

In order to investigate whether neuroplastic changes in stroke patients occur in response to an intense experimental rehabilitation program, we conducted the following methodological steps:

We scanned 10 healthy subjects to establish the protocol for tract reconstruction and to assess the variability. The subjects had to be scanned on two timepoints, since our aim is not only to investigate variability between subjects, but also between scans (variability between scans on two different timepoints) and between sequences (variability between sequences that were made in one scan session).
Then, a group of 5 patients who match our criteria was recruited (see 8.4). Their motor performance was tested and they received a DTI scan once as baseline scan.
In the following, each patient received a rehabilitation treatment, consisting of transcranial direct current brain stimulation (tDCS) and motor training of the affected limb. After the treatment period, the patients were tested again regarding their motor performance and underwent a second DTI scan in order to perform a pre-post-treatment comparison. As a control group, 5 healthy age-matched subjects were scanned.

8.1 Healthy subjects

In order to establish a protocol for assessing the pyramidal tract and the alternate motor fibers, 10 healthy subjects were scanned (mean age: 31 years, SD: 11.8; 9 right-handed, 1 left-handed; 7 male, 3 female). 7 out of the 10 subjects were scanned twice in order to test scan-to-scan variability.
In addition to the 10 subjects who served as a basis for the development of the protocol, we furthermore included a group of five healthy subjects on whom the established protocol has been applied, serving as an age-matched control group to the patients (demographical data of the age-matched controls: 3 male, 2 female; mean age: 58.0, SD: 8.1; all right-handed). None of the subjects had a history of a neurologic or psychiatric disorder.

8.2 Patients

Five chronic stroke patients were enrolled in this study (4 male, 1 female; all right-handed, two with right hemisphere lesions, three with left hemisphere lesions; mean age: 63.6, SD: 13.8; mean time after stroke 19.5 months, SD: 16.5; see Table 1 for detailed patient information and Fig. 7 for details of lesion location). Patients were selected from our prospectively collected database of chronic stroke patients participating in experimental therapy trials based on the following inclusion criteria: (1) at least 6 months after their first ischemic stroke; (2) no previous or subsequent strokes; (3) significant motor impairment within the acute stroke phase as assessed by a Medical Research Council (MRC) strength grade of ≤3/5 in extensor muscles of the affected upper extremity. The study was approved by the institutional review board and all participants gave written informed consent.

Table 1: ***Demographical data of the patients.*** *F=female, M=male, R=right, UE-FM= Upper Extremity Fugl-Meyer Motor Impairment Score (see 8.5). The treatment took place between the two scans, UE-FM was assessed pre- and post treatment for each patient.*

Patient	Lesion Side	Hand.	Age	Sex	1st scan post stroke (months)	2nd scan post stroke (months)	Baseline UE-FM	Absolute change in UE-FM
1	R	R	77	F	10.6	12.1	41	7
2	R	R	53	M	6.0	7.5	27	11
3	L	R	72	M	46.0	47.8	54	7
4	L	R	71	M	22.3	23.4	53	6
5	L	R	45	M	12.4	12.7	45	4

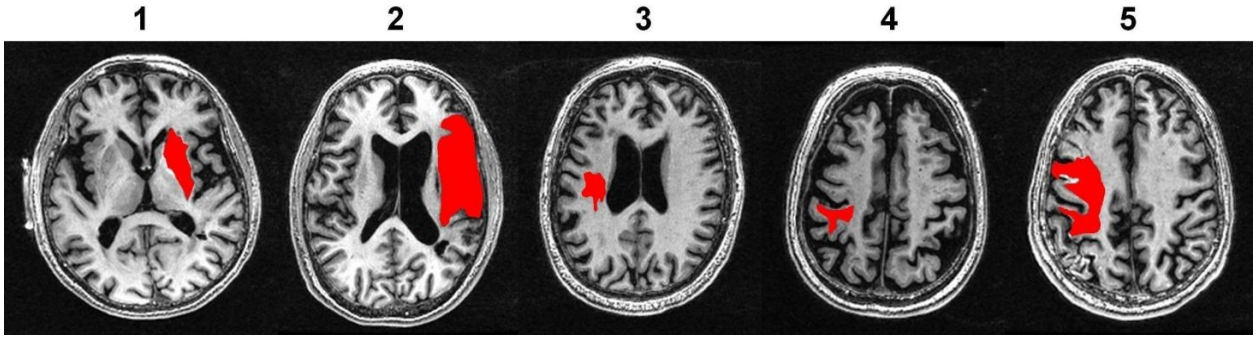


Figure 7: ***Lesion location of the five stroke patients.*** *Each slice shows the lesion at the level where the stroke lesion was depicted most prominently. Lesion areas are colored in red (numbers correspond to the patient numbering used in table 1)*

8.3 Motor assessment

Each patient underwent the Upper Extremity Fugl-Meyer assessment (UE-FM) before and after receiving the rehabilitation program. The UE-FM is designed specifically as an evaluative measure for assessment of recovery in the post-stroke hemiplegic patient and is frequently used in clinical trials to test motor impairment in stroke rehabilitation [42]. It consists of 30 items developed to assess motor function. Each item is scored on a 3-point ordinal scale (0 = *cannot perform*, 1 = *performs partially*, 2 = *performs fully*). The motor domain for the UE-FM includes items measuring movement, coordination, and reflex action about the shoulder, elbow, forearm, wrist and hand. The motor score ranges from 0 (hemiplegia) to a maximum of 66 points reflecting normal motor performance. [42, 43]

8.4 tDCS therapy

All patients participated in an experimental rehabilitation program which consisted of 2 x 5 sessions of daily bihemispheric tDCS for 30 minutes per session, with a current strength of 1.5 mA and simultaneous occupational therapy. Direct current was delivered using a Phoresor® II Auto stimulator (IOMED, Salt Lake City, UT) through two saline-soaked surface gel-sponge electrodes (16.3 cm^2 active area) [19]. In this bihemispheric stimulation montage, the cathode was placed over the contralesional motor region (either C3 or C4 according to the 10-20 EEG system) and the anode over the lesional motor region.

8.5 Behavioral therapy

An experienced occupational therapist utilized proprioceptive neuromuscular facilitation techniques, ensuring that patients received similar exercises (within the individual's physical capabilities). Typically, patients were trained to combine shoulder abduction, external rotation, elbow extension and forearm pronation into a single movement.

8.6 Imaging Data Acquisition and Analysis

All participants underwent MR scanning using a 3-Tesla GE scanner. Anatomical images were acquired using a T1-weighted, magnetization-prepared, rapid acquisition, gradient-echo (MPRAGE) volume acquisition with a voxel resolution of 0.93x0.93x1.5mm. DTI was performed using a diffusion-weighted, single-shot, spin-echo, echoplanar imaging sequence (TE1 = 86.9ms, TR = 10000 ms; FOV = 240 mm, matrix size 94x94, voxel size: 1.87x1.87x5.0 mm, no skip). Twenty-five non-collinear directions with a *b*-value of 1000 s/mm^2 and one image with a *b*-value of 0 s/mm^2 were acquired.

8.7 Diffusion Tensor Tractography

Diffusion tensors were calculated from all voxels within the brain using MedINRIA software version 1.5 (http://wwwsop.inria.fr/asclepios/software/MedINRIA/). Fractional anisotropy (FA) values were calculated within each brain voxel and fiber tracts were calculated by connecting adjacent voxels with similar principal eigenvectors, using a threshold FA value of 0.2 and a smoothness factor (a parameter ranging from 0 to 1 corresponding to the straightness of each fiber) of 0.2 for continuous fiber reconstruction. Only fibers with a length of >10 mm were included. These parameters are similar to those used by previous studies which applied a fiber assignment by continuous tracking algorithm [40, 44, 45].

8.8 ROI drawing protocol

We traced the PT and additional corticospinal motor fibers which also originate from the precentral gyrus, travel with the PT fibers, but separate at the level of the cerebral peduncles and take an alternate course through the posterior pons. We refer to this consortium of tracts as alternate motor fibers (aMF) [1] as described in chapter 5.1. In order to reconstruct the tracts, three ROIs were drawn on the FA and color-coded axial slices to reconstruct the PT and aMF as shown in Figure 8, while only those fibers that travelled through each of the three ROIs were included (all three ROIs are *logical* AND-functions). For PT-reconstruction, we drew the first ROI in the posterior limb of the internal capsule (PLIC), corresponding approximately to z=8 of a spatially normalized brain in Talairach space [46]. The second ROI included the precentral gyrus and its underlying white matter at the z-level of 50. In a third step, we drew a ROI in the anterior part of the cerebral peduncles at the level of z=-15. Cerebellar fibers were excluded by placing a region in the superior cerebellar peduncle and the middle cerebellar peduncle and applying a *logical* NOT-function.

For aMF reconstruction, the ROIs in the precentral gyrus and the PLIC were identical to the ROIs used for PT-reconstruction. However, the third ROI was placed in the posterior pons (tegmentum pontis) at the level where the superior cerebellar peduncle is visualized (z=-24).

For further analysis, we extracted the following parameters for both hemispheres: (1) FA-value, (2) $\lambda 1$ (axial diffusion along the principal direction of diffusion), (3) λ_2 and λ_3 (radial diffusion perpendicular to the principal direction, calculated by averaging the second and third eigenvalues $(\lambda_2+\lambda_3)/2$; hereafter referred to as $\lambda_{2,3}$) [2], and (4) Apparent diffusion coefficient (ADC).

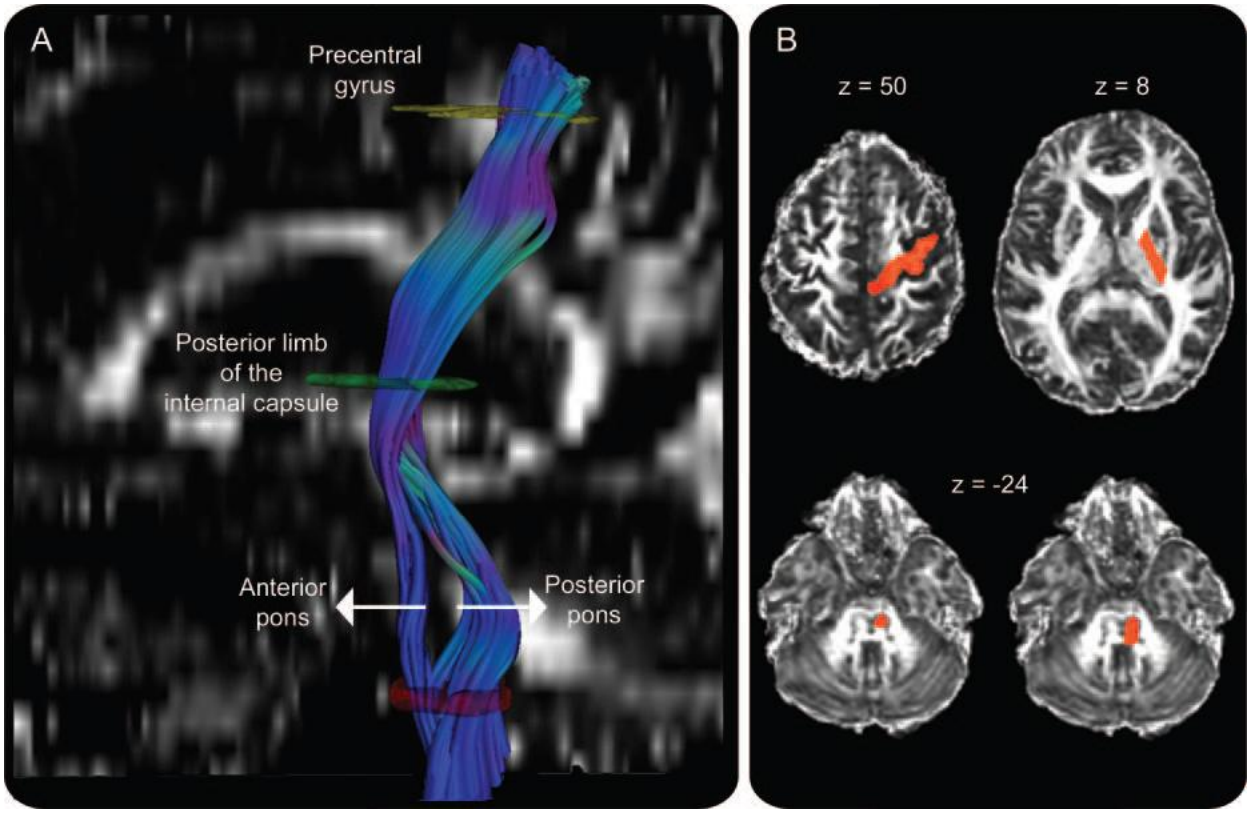


Figure 8: ***tract reconstruction of the PT and aMF. A:*** *The descending tracts differentiate into two different components in the pons with an anterior component corresponding to the pyramidal tract (PT) and a posterior component consisting of alternate or supplementary motor fibers (aMF).* ***B:*** *Examples of regions of interest (ROIs), overlaid onto a normalized fractional anisotropy image. The cortical ROI encompassed the precentral gyrus and its underlying white matter (z = 50). The ROI encompassing the posterior limb of the internal capsule was drawn at z = 8 and the pontine ROIs were drawn just below the superior cerebellar peduncle at approximately z= -24 (Coordinates are given in Talairach space). Adapted from Lindenberg et al., 2009 [1].*

8.9 DTI Data Analysis

In order to assess intra- and inter-rater reliability, one rater performed the measurements in the healthy subjects twice, remaining blind to the first set of measurements. The same subjects were measured a third time by a different rater who was blind to the results of the two sets done by the first rater. Intra- and inter-rater reliability was calculated using the intraclass correlation coefficient (ICC). For ICC calculation, a consistency definition for mixed effect designs was used. Results applied to single measurements (ICC (B,1)) for mixed effects designs.

In order to compare the variability in 10 healthy subjects to each other in respect to inter-subject, inter-session and intra-session variability, we used the coefficient of variation (CV) which is commonly used as a measure of variability [24, 47] (CV=standard deviation/mean x 100).

For the comparison of the patients and the control group, we conducted a cross-sectional comparison between groups for each parameter to assess between-group differences at baseline using a two-sample t-test.

We then compared post-intervention values to pre-intervention values in all parameters to investigate longitudinal changes within each hemisphere separately, using a paired t-test.

Furthermore, we calculated asymmetry indices (AI) for DTI-derived measures between the patients' lesional and contralesional hemispheres and between the controls' right and left hemispheres. AIs were calculated by equation 3 [38]:

$$\mathrm{AI} = \frac{\mathrm{P(right)} - \mathrm{P(left)}}{\mathrm{P(right)} + \mathrm{P(left)}}$$

Equation 2: Asymmetry index in healthy subjects

Accordingly, we used the following equation for the stroke patients:

$$\mathrm{AI} = \frac{\mathrm{P(affected)} - \mathrm{P(unaffected)}}{\mathrm{P(affected)} + \mathrm{P(unaffected)}}$$

Equation 3: Asymmetry index in stroke patients

P represents any of the four parameters determined (FA, λ_1, $\lambda_{2,3}$, ADC). Values for asymmetry may range from -1 to +1. A negative AI indicates higher values on the left/contralesional side, while a positive value means an asymmetry towards the right/lesional side. “0” represents perfect symmetry. Using the AI, we then performed a group-comparison to assess differences in hemispheric asymmetry at baseline using a two-sample t-test; longitudinal within-group changes in asymmetry throughout the period of recovery in patients were investigated using a paired t-test.

In order to compare the magnitude of variability in stroke patients throughout recovery to variability in the control group, CV was calculated based on the pre- and post-treatment scans for the five patients and on the first and second scans for the five healthy controls. CVs were calculated for each individual separately, then averaged across the group for each hemisphere.

9. Results

9.1 Intra-/inter-rater reliability of the tract reconstruction

Reproducibility was calculated by the Intraclass Correlation Coefficient (ICC). ICC values of <0.20 indicate slight agreement, 0.21-0.40 fair, 0.41-0.50 moderate, 0.60-0.79 substantial and 0.80-1.00 excellent agreement between ratings [48].

Averaged ICC values across parameters showed high test-retest agreement for intra-rater reliability. Inter-rater agreement was high concerning the pyramidal tract and was substantial with regard to the alternate motor fibers (see table 2) Figure 9 shows ICC values for each parameter.

Table 2: ***Intra-/inter-rater reliability.*** *Intraclass Correlation Coefficient (ICC) averaged across the parameters, values in parentheses represent standard deviations.*

	Pyramidal tract		Alternate motor fibers	
	Intra-rater reliability	Inter-rater reliability	Intra-rater reliability	Inter-rater reliability
Mean ICC	0.99 (0.01)	0.83 (0.11)	0.99 (0.01)	0.71 (0.16)

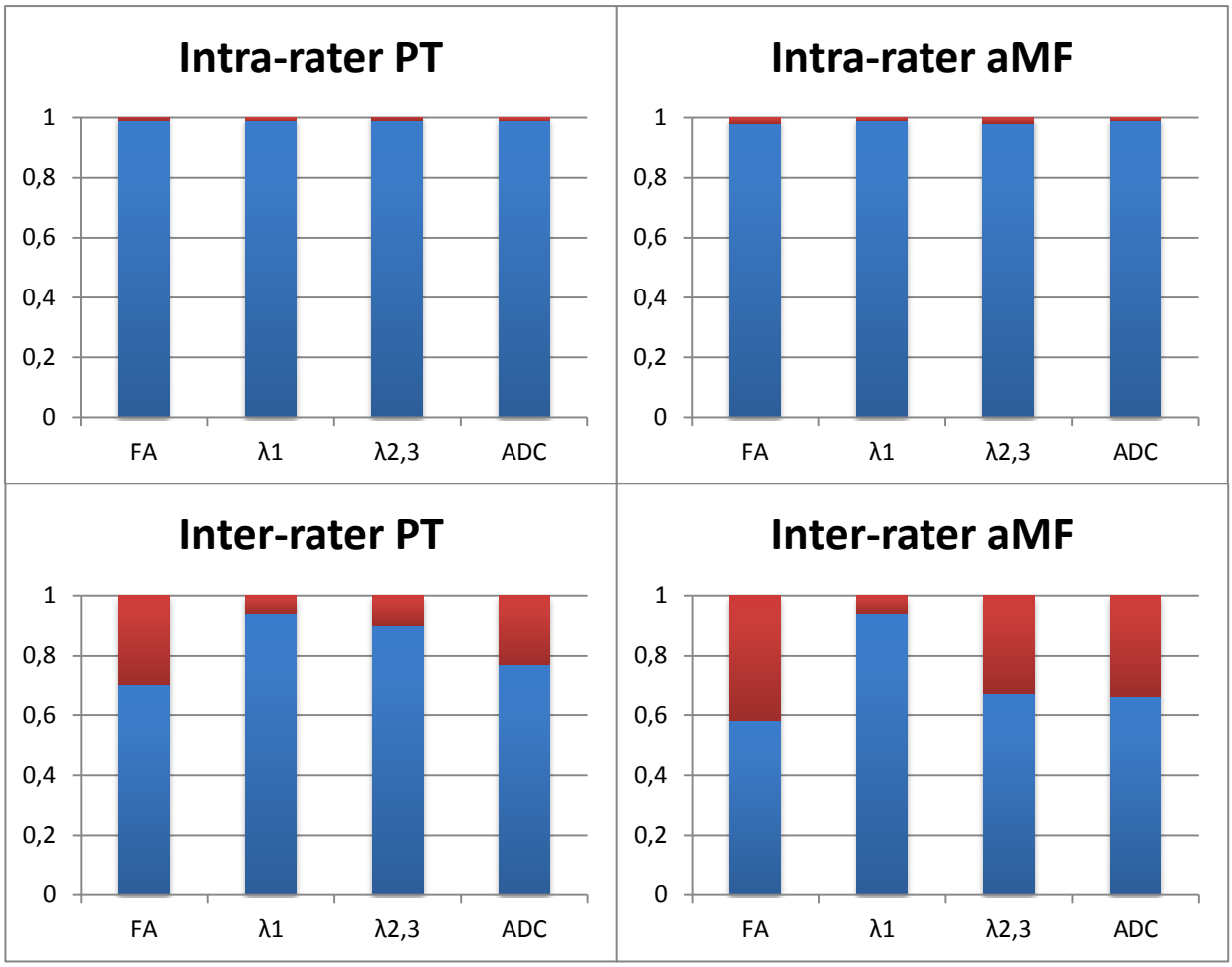


Figure 9: ***Intraclass correlation coefficient for inter-rater and intra-rater reliability.*** *Blue bars indicate the actual value of ICC of each parameter, red bars show the magnitude of the value that would have been necessary to achieve perfect agreement (ICC=1).*

9.2 Variability in 10 healthy subjects

Using healthy subjects we calculated the variability between the following categories: variability between subjects *(inter-subject variability)*, between scans *(inter-session variability)* and between sequences i.e. scans which have been collected within one scan-session; *intra-session variability).*

As expected, in all parameters, variability between subjects was found to be the highest, reflecting anatomical differences between subjects: in the PT, the inter-subject CV significantly differed from the inter-session CV (all ps≤0.01, ts(26)≤-3.6) and intra-session CV (all ps≤0.01, ts(26)≤-3.5). Similarly in aMF, inter-subject CV was significantly higher than inter-session CV (all ps≤0.01, ts(26)≤-2.7) as well as intra-session CV (all ps p≤0.02, ts(26)≤-2.5). Figure 10 shows the distribution of CVs. However, no significant differences could be detected between in inter- and intra-session CVs. This finding raises the assumption that physiologic microstructural changes or other influencing factors that could appear between two timepoints perish in the noise of DTI-measurements, at least in small sample sizes of n=5. Further considerations are described in the discussion section.

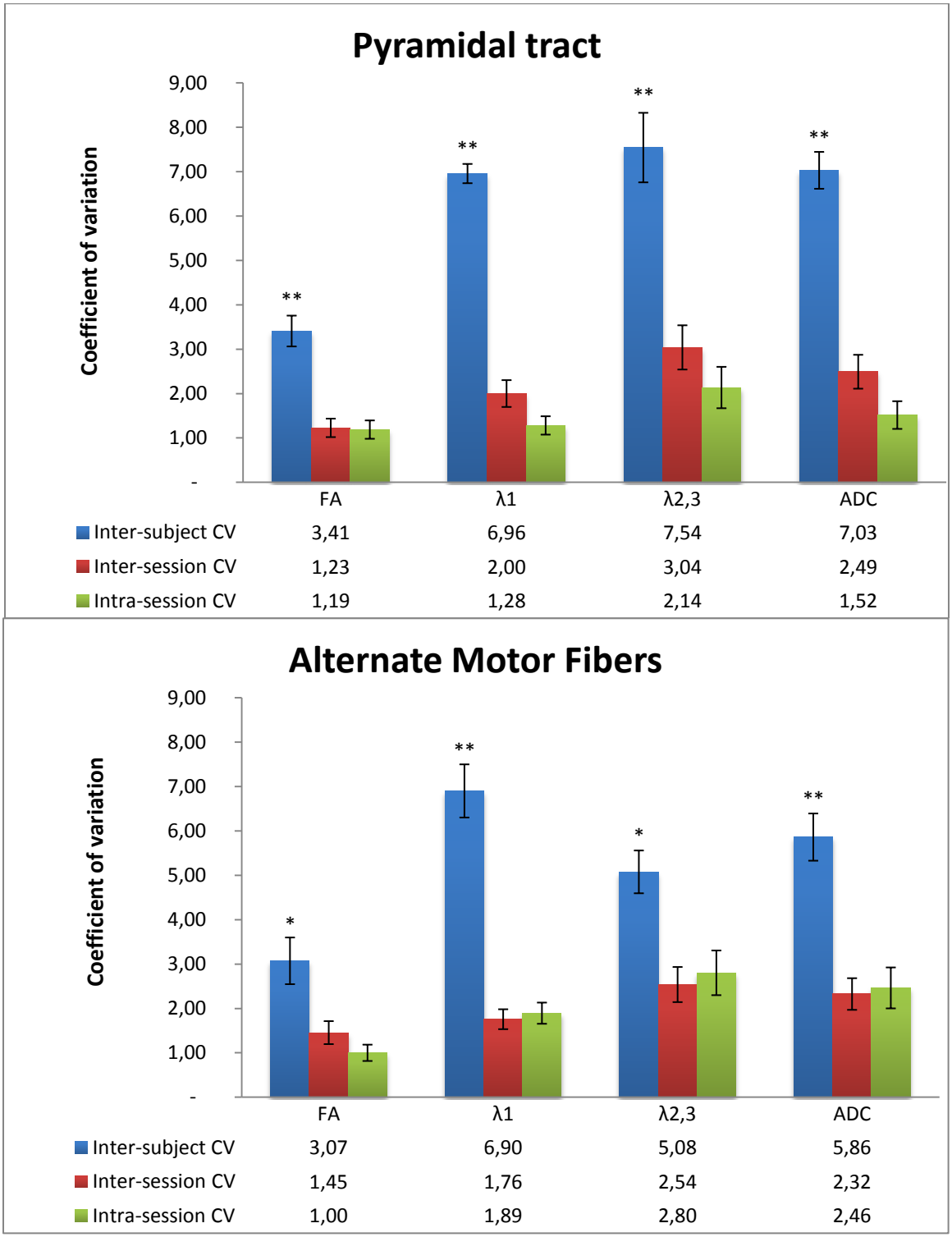


Figure 10: ***Inter-subject, inter-session and intra-session variability.*** *Variability between subjects was significantly higher than both inter- and intra-session variability, which were not significantly different from each other. Error bars indicate standard error of the mean. *: p<0.05; **: p<0.01*

9.3 Behavioral data of the patient group pre- and post-therapy

Each patient received an UE-FM assessment both before and after the treatment. A comparison of UE-FM scores pre-therapy to UE-FM scores post-therapy using a paired t-test revealed a significant increase of motor scores post-therapy ($p<0.01$; $t(4)=-6.1$) as shown in figure 11.

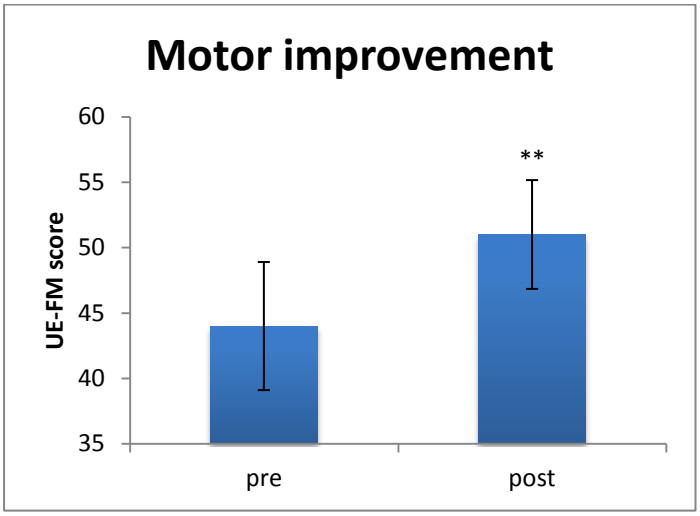


Figure 11: ***pre-post comparison of the motor skills.*** *The bars indicate the UE-FM scores pre- and post therapy averaged across the patient group. Error bars indicate standard error of the mean. **: p<0.01*

9.4 Between-group differences of DTI-derived measures at baseline

In a cross-sectional comparison, we found significant differences between the group of chronic stroke patients and the control-group in various parameters: Axial ($\lambda 1$) and radial ($\lambda_{2,3}$) diffusion as well as ADC were significantly increased in stroke patients on the lesional hemisphere in the PT (all *p*s<0.05); increased axial (trending toward significant, $p=0.07$) and radial diffusion ($p=0.01$), as well as ADC ($p=0.01$) were found in aMF (see table 3). The contralesional hemisphere showed no significant differences to healthy controls.

Table 3: ***Between-group differences at baseline.*** *Comparison of chronic stroke patients' lesional hemisphere (n=5) to healthy controls (left + right hem., n=10; two-sample student's T-test) at baseline.*

	Stroke patients (n=5)		**Healthy controls** (n=10)				
Pyramidal tract	Mean	SD	Mean	SD	df	t	p
FA-value	0.43	0.06	0.48	0.02	13	-2.2	0.04
λ_1	1.33	0.06	1.22	0.09	13	2.4	0.03
$\lambda_{2,3}$	0.77	0.06	0.66	0.02	13	3.2	0.01
ADC	0.56	0.05	0.45	0.02	13	3.5	0.01
Alternate motor fibers							
FA-value	0.44	0.05	0.48	0.03	13	-1.9	0.08
λ_1	1.29	0.06	1.20	0.09	13	1.9	0.07
$\lambda_{2,3}$	2.55	0.06	2.31	0.18	13	2.9	0.01
ADC	0.63	0.03	0.55	0.05	13	2.8	0.01

9.5 Longitudinal changes within the patient group

A comparison between pre- and post-intervention DTI-measures using a paired t-test did not detect significant changes in patients throughout the period of rehabilitation when investigating each hemisphere separately (all *p*s>0.10), i.e. a direct comparison of one hemisphere before treatment to the same hemisphere after treatment (see table 4 and 5. Figure 12 shows proportional changes throughout the period of treatment). However, when both hemispheres were taken into account using an asymmetry index, significant changes were detected (see 9.8).

Table 4: ***Longitudinal comparison of the lesional hemisphere*** *(pre-treatment vs. post-treatment state using a paired t-test).*

Pyramidal tract	N	**Pre**	SD	**Post**	SD	df	t	p
FA-value	5	0.43	0.06	0.43	0.06	4	0.3	0.80
λ_1	5	1.33	0.06	1.35	0.05	4	-0.5	0.66
$\lambda_{2,3}$	5	0.67	0.05	0.68	0.07	4	-0.7	0.54
ADC	5	2.66	0.10	2.71	0.13	4	-0.8	0.49
Alternate motor fibers								
FA-value	5	0.44	0.05	0.44	0.05	4	-1.6	0.18
λ_1	5	1.29	0.06	1.32	0.04	4	-1.1	0.33
$\lambda_{2,3}$	5	0.63	0.03	0.65	0.06	4	-1.0	0.36
ADC	5	2.55	0.65	2.62	0.12	4	-1.1	0.32

Table 5: ***Longitudinal comparison of the contralesional hemisphere*** *(pre-treatment vs. post-treatment state using a paired t-test).*

Pyramidal tract	N	**Pre**	SD	**Post**	SD	df	t	p
FA-value	5	0.47	0.02	0.48	0.29	4	-0.6	0.59
λ_1	5	1.29	0.04	1.27	0.04	4	1.1	0.33
$\lambda_{2,3}$	5	0.60	0.03	0.59	0.03	4	1.2	0.31
ADC	5	2.49	0.10	2.44	0.08	4	1.2	0.29
Alternate motor fibers								
FA-value	5	0.47	0.03	0.47	0.03	4	1.0	0.37
λ_1	5	1.23	0.03	1.20	0.04	4	-1.5	0.21
$\lambda_{2,3}$	5	0.57	0.02	0.56	0.03	4	2.1	0.11
ADC	5	2.37	0.06	2.31	0.09	4	2.0	0.12

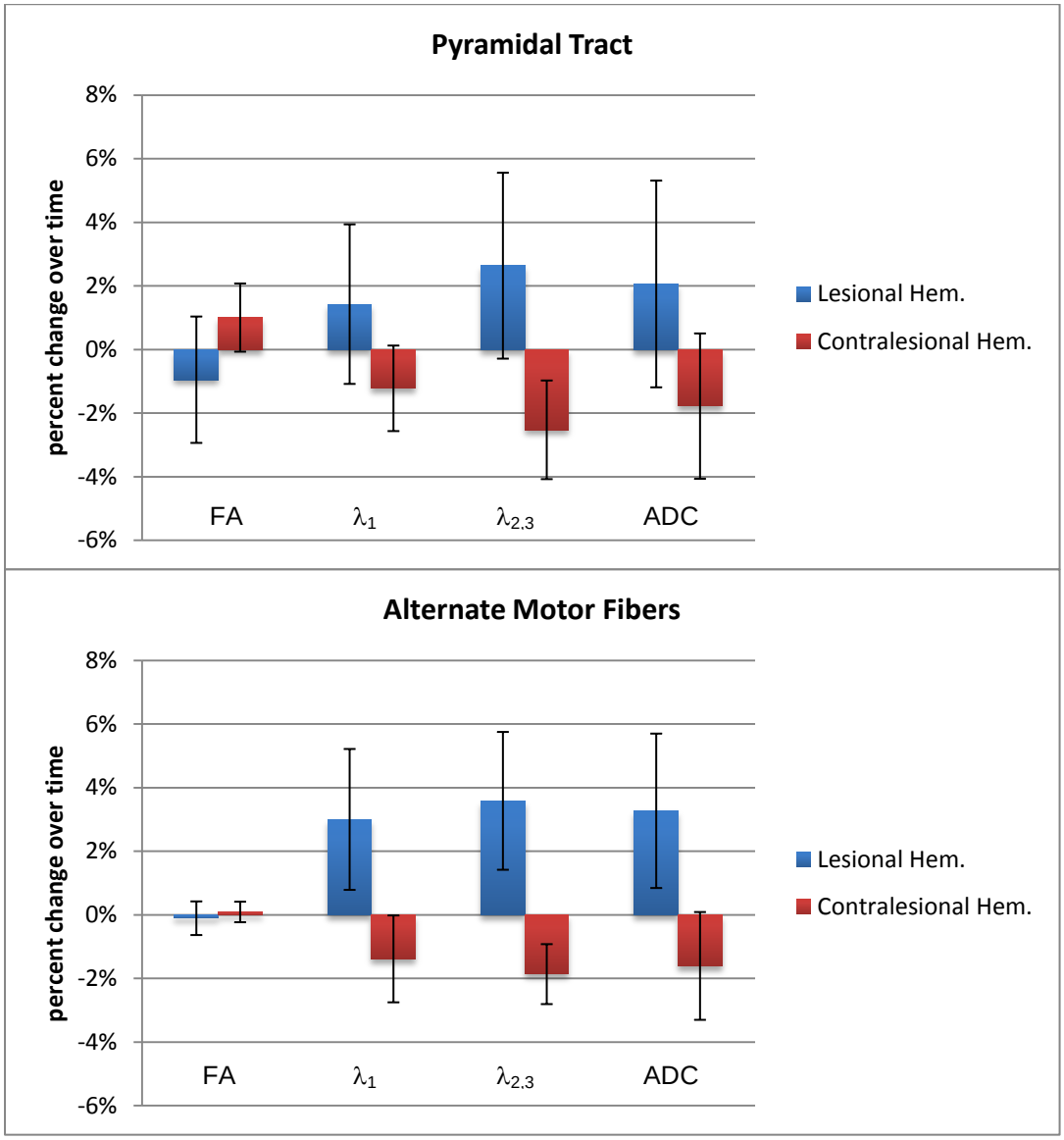


Figure 12: ***Proportional changes of diffusivity parameters in the patient group.*** *The longitudinal change (%) over the time of rehabilitation for each parameter is shown for the pyramidal tract and alternate motor fibers. Error bars indicate standard error of the mean.*

9.6 Changes in Asymmetry

9.6.1 Between-group differences of hemispheric asymmetry at baseline

In order to consider not only the lesional hemisphere *or* the contralesional hemisphere separately but to take the interaction of *both* hemispheres into account, the use of an asymmetry-index is useful. Negative scores indicate an asymmetry towards the left/contralesional hemisphere; positive scores indicate an asymmetry towards the right/lesional hemisphere.

In a comparison of the two groups at baseline, we found hemispheric asymmetry to be significantly different in the patient group compared to the controls: both PT and aMF patients showed significantly higher $AI_{\lambda 2,3}$ and AI_{ADC} (all *p*s<0.05) indicating higher diffusivity in the lesional tracts and showed trends in AI_{FA} (*p*s<0.10; see table 6).

Table 6: ***between-group differences of hemispheric asymmetry at baseline*** *(two-sample t-test).*

	Stroke patients (n=5)		**Healthy controls** (n=5)				
Pyramidal tract	Mean	SD	Mean	SD	df	t	p
FA-value	-0.05	0.05	0.00	0.01	8	-2.1	0.06
λ_1	0.02	0.02	0.00	0.01	8	1.5	0.18
$\lambda_{2,3}$	0.05	0.02	0.01	0.03	8	2.5	0.04
ADC	0.03	0.01	0.00	0.02	8	2.7	0.03
Alternate motor fibers							
FA-value	-0.03	0.03	0.00	0.02	8	1.9	0.10
λ_1	0.02	0.02	0.00	0.01	8	1.9	0.09
$\lambda_{2,3}$	0.05	0.02	0.01	0.03	8	3.6	0.01
ADC	0.04	0.01	0.00	0.02	8	3.1	0.02

9.6.2 Longitudinal within-group changes in asymmetry indices

In both PT and aMF, asymmetry indices of diffusivity parameters changed significantly within the group of stroke patients when pre-intervention values were compared to post-intervention values using a paired t-test (see figure 13 for longitudinal AI-changes). According to the observed alteration in diffusivity, asymmetry indices of axial diffusion, radial diffusion as well as ADC significantly increased through the period of rehabilitation in aMF (λ_1: p=0.02; t(4)=-3.8; $\lambda_{2,3}$: p=0.04; t(4)=-2.9; ADC: p=0.03; t(4)=-3.3); in the PT, the increase of AIs did not reach significance (see table 7). Apparently, although this increase in asymmetry means an intensification of the baseline differences between patients and controls, it is paralleled by an improved motor outcome. Potential explanations for this phenomenon are discussed in Chapter 10.6.

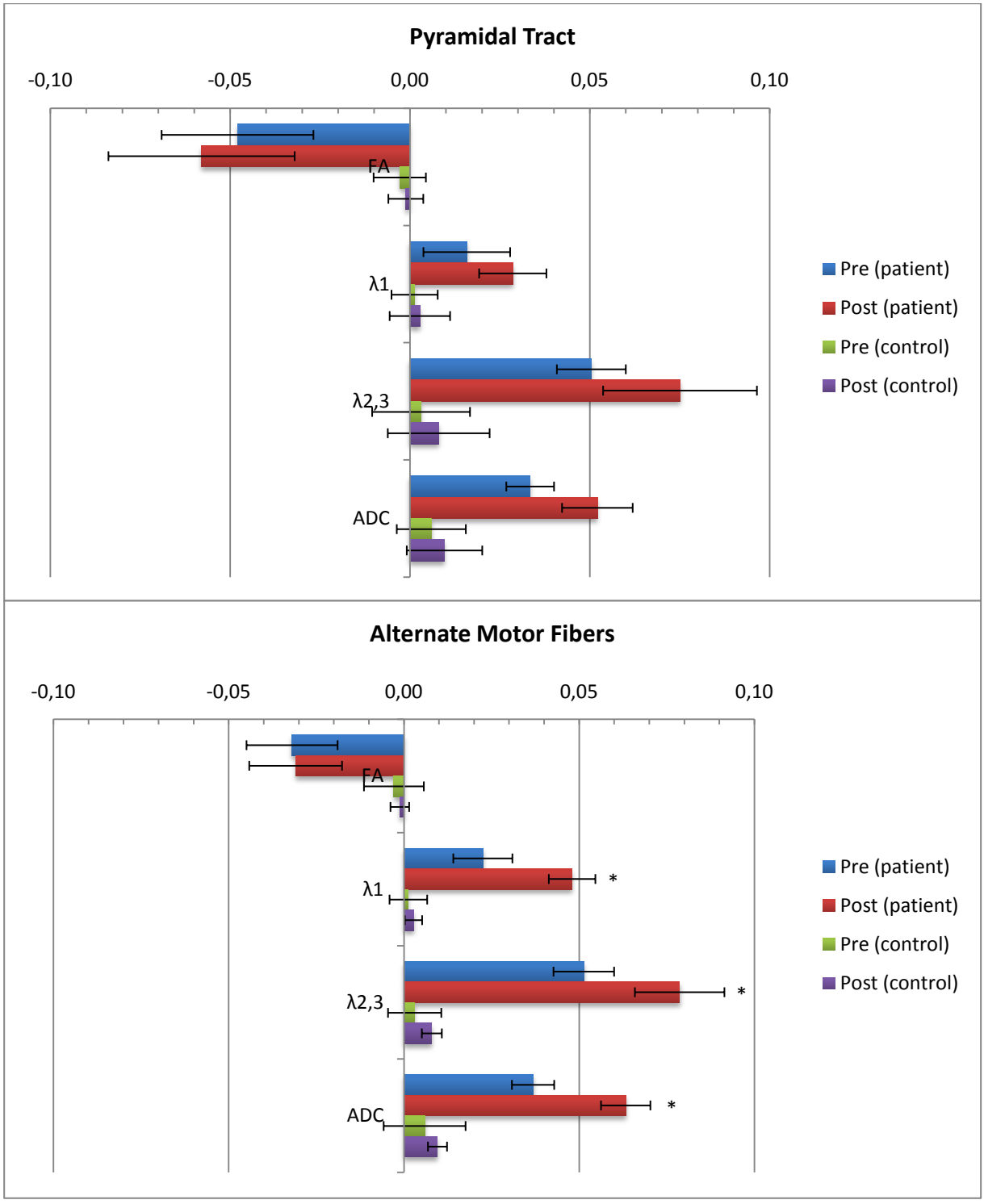


Figure 13: ***Longitudinal changes in asymmetry index (AI).*** *A pre-post comparison of AIs in patients and controls. Negative AI indicates an asymmetry towards the left/contra-lesional hemisphere, positive values an asymmetry towards the right/lesional hemisphere, whereas "0" indicates perfect symmetry. Values represent mean AI for each group. *: p<0.05*

Table 7: ***Asymmetry changes throughout recovery in patients.*** *A longitudinal within-group analysis of the patient group using a paired t-test, Asymmetry Indices (AI) averaged across the group.*

Pyramidal tract	N	**AI Pre**	SD	**AI Post**	SD	df	t	p
FA-value	5	-0.05	0.05	-0.06	0.06	4	0.8	0.46
λ_1	5	0.02	0.02	0.03	0.02	4	-1.6	0.18
$\lambda_{2,3}$	5	0.05	0.02	0.08	0.05	4	-1.6	0.19
ADC	5	0.03	0.01	0.05	0.02	4	-2.3	0.09
Alternate motor fibers								
FA-value	5	-0.03	0.03	-0.03	0.03	4	0.0	1.00
λ_1	5	0.02	0.02	0.05	0.01	4	-3.8	0.02
$\lambda_{2,3}$	5	0.05	0.02	0.08	0.03	4	-2.9	0.04
ADC	5	0.04	0.01	0.06	0.02	4	-3.3	0.03

9.7 Comparing healthy controls and patients in variability measures

Using a two-sample t-test, we compared the patients to the controls with regard to their CV (coefficient of variation = standard deviation/mean x 100). On the lesional hemisphere, CV based on pre- and post-treatment values in the patient group exceeded the CV of healthy control subjects based on scan 1 and scan 2 in the following parameters: in the PT we found higher CVs in FA (p=0.05) λ_1 (p=0.01) and ADC (p=0.05) and similar trends for the λ_1 and ADC in aMF (see table 8). No significant differences were found when the nonlesional side was compared to healthy controls.

Table 8: ***Between-group comparison of coefficients of variation.*** *Comparison of patients vs. controls using a two-sample t-test (CVs calculated on the basis of pre-post values for the patients (n=5) and on scan1/scan2 values for the controls (right+left hemisphere; n=10));*

Pyramidal tract	**Patients**	SD	**Controls**	SD	df	t	p
CV FA	2.5	1.7	1.1	0.8	13	2.12	0.05
CV λ_1	2.9	2.4	0.7	0.6	13	2.84	0.01
CV $\lambda_{2,3}$	3.4	1.7	1.7	1.0	13	1.51	0.16
CV ADC	3.1	2.6	1.3	0.7	13	2.21	0.05
Alternate motor fibers							
CV FA	0.8	0.3	1.7	1.7	13	1.14	0.28
CV λ_1	3.3	2.9	1.3	1.2	13	1.83	0.09
CV $\lambda_{2,3}$	3.4	2.8	2.0	1.3	13	1.34	0.21
CV ADC	3.3	2.6	1.6	1.1	13	1.80	0.09

10. Discussion

The discussion will focus on the findings in the order as they were presented in the Results section. First, it will concern the methodical aspects, namely the reproducibility of our tract reconstruction as well as the meaning of our variability assessments in healthy subjects. After that, it will deal with the therapy and the resulting motor improvement along with the current theories on the beneficial effects of tDCS. A discussion of methodical questions will be followed by a review of the actual differences in diffusivity: explanations for the differences we found when comparing patients to controls at baseline, and possible explanations will be put forward as to why the differences at baseline intensified within the patients during the period of rehabilitation therapy – paralleled by motoric improvement. Here, the question that shall be addressed is which general mechanisms causing the intensification could be considered: pathologic vs. salutogenetic factors. Finally, more specific factors will be discussed e.g. rewiring, neuronal sprouting and its consequences on diffusivity and other possible explanations such as long-term influences of tDCS stimulation on diffusivity in the brain.

10.1 The drawing protocol: a reproducible tool for tract-reconstruction

The drawing protocol which was developed for this study turned out to be robust both to test-retest procedures performed by one rater and two independent raters. Similar to the findings of previous studies, reproducibility by one rater was higher than the inter-rater reproducibility [49, 50]. However, the description of the protocol and the landmarks it uses are precise enough to achieve substantial to high agreement between independent raters which makes it suitable for future studies addressing similar questions.

10.2 Potential explanations for the variability pattern in healthy subjects

A simple way of gathering information about the impact of microstructural changes and interfering factors on DTI-measurements is to measure both the variability between scans of different timepoints where microstructural changes and other influencing factors could have happened and compare this variability to the variability between scans made in a single scan-session (=sequences) where microstructural changes or position-dependent artefacts are unlikely to happen. This is the main reason we assessed these two measures along with the intersubject variability with the following result: Variability between subjects was higher than both variability between scans and variability between sequences. A similar relation between inter-subject and inter-session variability was found by previous studies [37, 50]. This relation can be explained by anatomical differences between the subjects. For example, some subjects may have different diffusion patterns in their brains according to the extent of training of the concerned brain region. The training of certain brain regions like the motor system is paralleled by diffusivity changes and can be due to intensive motor training such as playing an instrument [41].

However, no significant difference could be found when comparing inter-session and intra-session variability. Our hypothesis of a higher inter-session variability relative to intra-session variability was based on the assumption that the latter is not as sensitive to certain factors that may influence the inter-session variability: potential structural changes in the brain happening between the first and the second scan, potential micro-strokes which remain clinically unapparent but may alter diffusion properties in the brain, different positions of the subject during the different scans etc.

The results of the present study do not support this hypothesis. The fact that we did not detect significant differences comparing these two variability measures does not definitively rule out the possibility that these factors influence the variability between scans, but it shows that the variability of the DTI procedure itself is not able to capture factors as mentioned above given a group size of n=10.

10.3 Motor improvement as a response to tDCS- and behavioral therapy

In response to our therapy, stroke patients showed a significant improvement of motor skills over the course of rehabilitation. Behavioural therapy in terms of peripheral stimulation using neuromuscular facilitation techniques as applied in routine rehabilitative therapy or other sensorimotor activities may enhance the effects of tDCS-therapy [19]. The beneficial effects of tDCS alone have been shown by previous studies [51, 52] while other studies suggest an amplifying effect of combining brain stimulation with simultaneous peripheral stimulation [53].

The theory of the beneficial effect of tDCS is based on its modulating effect of the interhemispheric interaction; studies using functional magnetic resonance imaging (fMRI) have shown an increased bihemispheric activation pattern in patients with acute stroke when the affected limb is moved (progressively lateralizing in the chronic stage of the stroke) [54, 55]. An early re- or overactivation of the lesional hemisphere was associated with better behavioral results of the patients [54, 55]. The role of the contralesional activation in stroke recovery still remains uncertain. The theory for tDCS-therapy in stroke is the following: blocking or depressing the contralesional hemisphere results in improved motor outcome, because it is thought to influence the imbalance of interhemispheric inhibition [19]. The assumed model in stroke patients is a higher inhibition of the lesional hemisphere by the contralesional hemisphere than vice versa; the inhibition of the contralesional hemisphere by the lesional hemisphere is not at a similar level as it is being inhibited itself (figure 14). By using anodal stimulation on the lesional side and/or cathodal stimulation on the nonlesional side, this inhibitory imbalance may be partly restored [19].

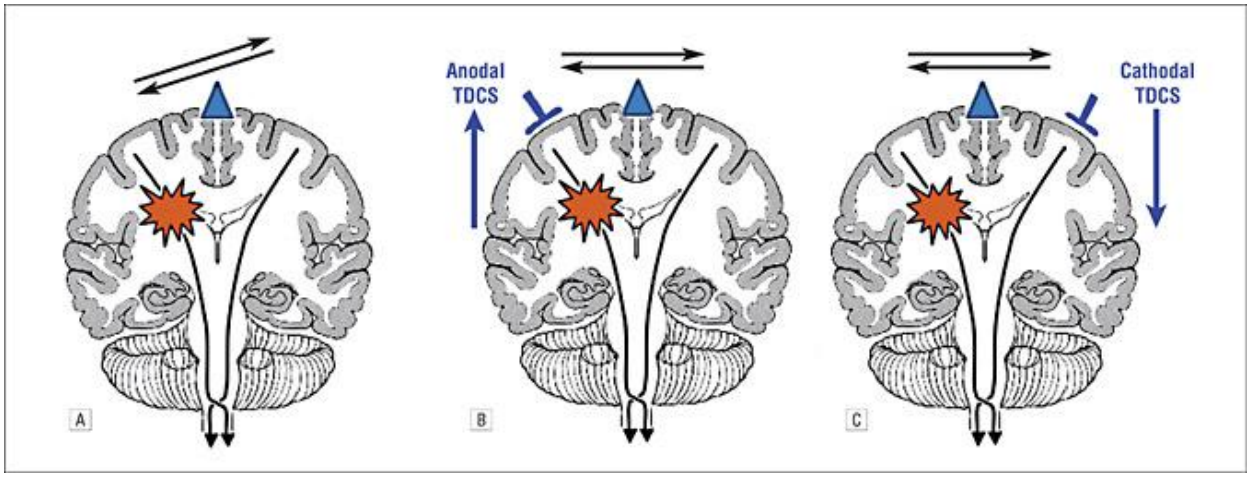


Figure 14: ***Brain model of imbalanced interhemispheric inhibition.*** *The balance of interhemispheric inhibition becomes disrupted after a stroke (A). This leaves the healthy hemisphere in a position in which it could exert too much of an unopposed or imbalanced inhibitory effect on the lesional hemisphere and possibly interfere in the recovery process of the lesional hemisphere. There are 3 possible ways to ameliorate this imbalance, namely, up-regulation of the excitability in the lesional hemisphere (B) down-regulation of the excitability in the contralesional hemisphere (C), or a combination of A and B in terms of simultaneous anodal and cathodal stimulation (dual stimulation) as used in this study. Adapted from Schlaug et al., 2008 [19].*

The imbalance of inhibition described above is one model of the bihemispheric interaction. Other studies [56-58] suggest a compensatory role of the contralesional (ipsilateral) hemisphere and it has been shown that parallel to the finger-movement of the unaffected hand in stroke patients, regional cerebral blood flow increased significantly in contralateral primary sensorimotor cortex and in the ipsilateral cerebellar hemisphere. However, when the fingers of the recovered hand were moved, significant regional increase of cerebral blood flow occurred in both contralateral and ipsilateral primary sensorimotor cortex and in both cerebellar hemispheres [59]. This finding suggests a compensatory role of the contralesional hemisphere and may also contribute to the fact that we found increasing asymmetry between hemispheres over the course of rehabilitation (see 10.5 and 10.6). However, the question of how the contralesional motor system influences the motor recovery is still to be answered and requires further investigation.

10.4 Between-group differences at baseline reflecting axonal degradation

In accordance with previous studies [60, 61], we found differences when comparing the patients' lesional side to healthy controls at baseline. A between-group comparison at baseline showed higher diffusivity in patients as compared to controls in both lesional tracts: an increased ADC and a decreased FA-value due to a higher diffusivity, in particular a radial diffusion with a relatively stronger increase over axial diffusion. This difference in diffusion is thought to be caused by the degradation of axonal membranes in the acute phase and furthermore the degradation of myelin sheath in the subacute phase, reflecting a phenomenon which occurs after stroke: wallerian degeneration (WD; [34-36]) which describes the degeneration of an axon after it is separated from the cell's nucleus and which follows a stereotypical time course. The so called acute axonal degeneration happens very quickly (approximately 30 min) after the event of injury [62]. Thus, the short term effects of WD will not affect this study, since only stroke patients in a chronic state were included. Although long-lasting processes have been reported [63], a recent DTI study showed that initial changes in diffusivity due to WD stabilize after 3 months at a certain degree of increased diffusivity [36]. Hence, the baseline differences, but not the longitudinal changes are presumably caused by WD since our patients were included in the study with much longer intervals than 3 months between stroke and the first MRI-scan; the smallest interval was 6 months after stroke.

10.5 General considerations on longitudinal changes within the patient group

Our main findings regarding longitudinal changes in patients are as follows: When each hemisphere was investigated separately, i.e. one hemisphere pre-therapy in comparison to the same hemisphere post-therapy, a longitudinal within-group analysis did not detect significant changes throughout the period of rehabilitation. However, with regard to hemispheric asymmetry, we found both significant differences at baseline between the groups and longitudinal changes within the patient groups: at baseline, patients showed a higher diffusivity in lesional tracts as compared to the contralesional side, which increased significantly over the course of our intervention;

When discussing the fact that we did not find longitudinal changes in a pre-post comparison for each hemisphere, several things have to be taken into account that may have contributed to this result: first, our sample size was relatively small, so even with the trends going into the assumed direction, significance was not reached. Second, the magnitude of changes pre- to post-therapy is very small, hence, a tool has to be found which is capable of detecting these changes through other ways, e.g. by taking both hemispheres into account. Considering both hemispheres at the same time may be important when addressing the question of stroke rehabilitation since the contralesional hemisphere is involved in reorganization and recovery after stroke [56-58]. In our case, the asymmetry index reported significant results. This is mainly due to the fact that the changes we observed go in opposite directions in each hemisphere. Despite the fact that the involvement of the contralesional hemisphere in stroke recovery is commonly assumed and that the interaction of both hemispheres plays a role in stroke-rehabilitation, the way in which the contralesional hemisphere interferes with the lesional hemisphere has not yet been clarified. Possible interpretations of the diffusivity changes that we observed will be presented in the following section, 10.6.

We also have to discuss the question of whether the longitudinal changes that emerged parallel to the motor improvement are either a response to our therapy or just a further manifestation of the pathological factor that caused the differences: we found differences between the group of patients and the controls; all patients showed a similar diffusion pattern (high diffusivity on the lesional side, low contralesional diffusivity) compared to relatively equal distribution of diffusivity in controls. These differences we observed at baseline became more prominent in the same direction over the course of recovery which leaves the following possible explanations for this further increase:

1) The pathological process that caused the diffusivity-shift such as Wallerian Degeneration may have been responsible for the further shift into the same direction: one may assume that the progressive degradation of myelin sheath, as it happens in wallerian degeneration, leads to increased radial diffusion, as has been shown in previous studies [36].

However, this factor might only – if at all – play a minor role in our study, since it has been shown that changes in diffusivity due to WD stabilize after about three months post stroke [36]; the patients enrolled in our study, however, are all in a chronic phase with a mean time post stroke of 19.5 months where the effects of WD could not be detected by DTI anymore. Consequently, the increase in the differences we found may be due to other influencing factors.

2) The further intensification of the diffusivity pattern may also be interpreted as a salutogenetic response to the intervention: the patients were all in a chronic state; hence, we can assume that even without a therapeutic rehabilitation program, natural recovery happened or is still happening in the brain. This kind of natural recovery may have caused a certain diffusivity pattern in the interval between stroke and our 1st scan, leading to differences at baseline and became amplified by the intensive rehabilitation program we used. In this case, the increasing asymmetry in the brain may be understood as a reflection of a compensational attempt, where the patients showed a motor improvement, although the diffusivity pattern goes off the path of healthy controls.

It is probable that we have a certain overlap between 1) and 2) in the respect that the effects of WD may have contributed to the baseline differences, however, it cannot explain the longitudinal changes for the reasons mentioned above. Apparently, the consequences of stroke and regenerative processes have an impact on diffusivity which goes in the same direction as WD to a certain extent.

10.6 Theories for diffusivity changes in the lesional/contralesional hemisphere

The increase of diffusivity in the lesional hemisphere may reflect various simultaneous mechanisms. Changes in microstructure may have changed diffusion properties in a way reflected by our findings. Several mechanisms of stroke recovery have been reported which may occur at the same time:

1) Regeneration: neurons situated in periinfarct regions sprout new connections building novel projection patterns and newly born immature neurons migrate to periinfarct regions [64].

2) Reorganization: remapping of representations previously situated in lesional areas to nonlesional areas such as periinfarct regions or the contralesional hemisphere [19]. Peri-infarct reorganization in non-humans can occur after focal cortical lesions in the primary motor cortex, which may contribute to motor recovery by allowing other areas near the lesioned area to gain control of the weak body part [65, 66]. In humans, in a group of four stroke patients who had a small lesion located in the primary motor cortex (M1), recovery of finger movements was associated with a dorsal shift of the cortical activation areas within M1 over a period of two years [67]. Additionally, the premotor cortex has been suggested to play a major role in motor recovery after stroke [68]. Dancause et al. provided evidence for altered trajectories of axons originating from the premotor cortex, which is partly included in our ROI drawing protocol since the precentral gyrus consists of both the M1 and parts of the premotor cortex [69].
In response to our therapy, the remodelling might even be enhanced. This assumption raises the question of how this is reflected in diffusivity. To answer this question, one must consider the way in which DTI-derived measures are computed: The tract-based statistics takes every voxel into account, which has an eigenvector similar to the adjacent voxel. This means that the values we are considering are averaged across these entire tracts. If sprouting on the cortical areas is involved, we would expect a rather isotropic diffusion in these cortical and peri-infarct regions, whereas other regions, such as the lower parts of the tract might show enhanced myelination, leading to anisotropic diffusion. Taking these two factors into account, the result may be a polydirectional increase in diffusivity. The fact that λ_2 and λ_3 increased as well, which is

atypical for enhanced myelination, might also be due to altered permeability in axonal membranes at the non-myelinated parts of the axon (Nodes of Ranvier) in response to training as assumed by previous studies [41]. Membrane permeability might in this case weigh more than the degree of myelinization, as membrane permeability is generally thought to play a major role over myelinization in altering diffusivity [12, 70].

To clarify the question whether the way of computation explains the findings of this study, further investigation is needed in terms of a slice-by-slice analysis to see potential diffusion patterns according to certain tract portions in response to therapy.

In the longitudinal within-group comparison of hemispheric asymmetry, aMF showed more prominent results than the PT. An explanation for this finding might be that the lesional PT may have been damaged to an extent that did not allow plastic changes anymore; a compensatory role of aMF for motor recovery after stroke has been suggested previously based on neurophysiological [71, 72] and DTI studies [1]. However, while the path of pyramidal fibers is well known [3-5, 7], the course of what we termed “alternate motor fibers” (aMF) is not yet based on anatomical studies in humans [73, 74].

Finally, we also have to take into account that no studies have yet addressed the question of whether tDCS has long-term influences on diffusivity in the targeted area. If so, this may also explain the fact that the lesional hemisphere (anodal stimulation) showed opposite results (see figure 12) to the contralesional hemisphere (cathodal stimulation).

10.7 Comparing changes in patients to normal variability

Pre-post-CV of the patient group exceeded scan-to-scan CV of controls in FA, λ_1 and ADC in the PT and showed similar trends in aMF. However, in the comparison of FA between patients and controls in aMF, we found a relation which is contrary to what we expect and what the rest of the analysis showed: a (non-significantly) higher CV in controls than in patients. This finding shows us that the small group size in this study has to be taken into account when drawing conclusions. The statistical power is limited and further investigation is required. Nevertheless, despite the small sample size, the results of this study are relatively consistent with the exception of the above-mentioned parameter (FA) and suggest that structural plasticity as a function of motor-improvement is reflected in DTI-derived measures and may be used to evaluate therapeutic response to neurorehabilitation.

10.8 Limitations

As mentioned above, the major limitation of this study is the small sample size which provides only poor statistical power. Furthermore, our study design included healthy subjects as a control group. This represents a limitation in some regards: even though we have strong suggestions that the changes we observed are not substantially influenced by the effects of wallerian degeneration (see 10.5), we cannot exclude the possibility that other structural changes due to stroke (not due to our intervention) or wallerian degeneration, at least to a small extent, might have influenced the changes we found. Using a control group of stroke patients which does not receive the treatment would allow to address this issue. Another limitation of this study is the way of the DTI-derived measures were computed. As mentioned above, the tract statistics use data accumulated throughout the whole tract, i.e. changes in one direction in a certain part of the tract and changes in the opposite direction in another part of the tract may result in a tract statistic which only allows to evaluate the tract as a whole. In order to differentiate between certain patterns of diffusivity change within one tract, a slice-by-slice analysis is necessary.

11. Summary

Objective:

The aim of the study is to investigate potential neuroplastic changes in stroke patients as a response to an intense experimental rehabilitation program. The program consisted of combined brain stimulation and motoric training. With the use of diffusion tensor imaging, potential structural changes were investigated longitudinally in a pre-post-treatment comparison.

Methods:

Ten healthy subjects were scanned to develop the protocol for tract reconstruction and to investigate inter-subject, inter-session and intra-session variability. Seven out of the ten subjects were scanned again at another timepoint in order to assess inter-session variability.

Furthermore, five chronic stroke patients showing motor impairment underwent DTI before and after an experimental neurorehabilitation program including 10 days of transcranial direct current stimulation (30 min per day) and simultaneous occupational therapy (1 hour per day). A group of five age-matched healthy subjects served as control group to the patients. Fractional anisotropy (FA), apparent diffusion coefficient (ADC), and radial and axial diffusivity were determined for the pyramidal tract (PT) and alternate motor fibers (aMF). Indices of hemispheric asymmetry of these parameters were calculated for each patient.

Results:

In the group of healthy subjects, variability, expressed as coefficient of variation (CV) between subjects, was found to be the highest in all investigated DTI-derived measures: FA, axial diffusion (λ_1), radial diffusion ($\lambda_{2,3}$) and apparent diffusion coefficient (ADC). Inter-session- and intra-session CV, however, were not significantly different from each other.

A group comparison (two-sample t-test) between patients and controls at baseline showed significantly higher diffusivity on the lesional hemisphere in patients than in controls. In the patient group, the improvement in motor function was paralleled by significant longitudinal changes in hemispheric asymmetry for ADC as well as axial and

radial diffusivity due to enhanced diffusivity of lesional aMF post intervention. Changes in the patient group exceeded normal variability in the following parameters: FA, $\lambda_{1,}$ ADC in the PT and a trend for λ_1 and ADC in aMF.

Conclusions:

Effects of stroke recovery could be detected in tract asymmetries of diffusivity parameters. Structural plasticity as a function of motor improvement was reflected in DTI-derived measures and might be used to evaluate therapeutic response to neurorehabilitation. However, high variability of DTI-derived measures has to be taken into account when investigating changes of small magnitude.

Zusammenfassung

Zielsetzung:

Ziel der Studie ist die Untersuchung möglicher neuroplastischer Veränderungen bei Schlaganfallpatienten als Antwort auf ein intensives, experimentelles Rehabilitationsprogramm. Das Rehabilitationsprogramm besteht aus einer Kombination von Hirnstimulation und motorischem Training. Mit der Technik der Diffusions-Tensor-Bildgebung wurden mögliche strukturelle Veränderungen longitudinal untersucht, indem der Zustand vor und nach Therapie verglichen wurde.

Methodik:

Zehn gesunde Probanden erhielten eine Diffusions-Tensor-Bildgebung auf Basis derer das Protokoll für die Traktrekonstruktion entwickelt wurde. Weiterhin wurde damit auf die Variabilitäten zwischen Probanden (inter-subject variability), zwischen Bildgebungseinheiten (inter-session variability) und zwischen einzelnen Sequenzen innerhalb einer Einheit (intra-session variability) untersucht.

Fünf Schlaganfallpatienten im chronischen Stadium, die motorische Defizite aufwiesen, erhielten vor und nach einer experimentellen neurorehabilitativen Therapieeinheit eine Diffusions-Tensor-Bildgebung. Die Therapieeinheit erstreckte sich über zehn Tage und umfasste eine Hirnstimulation in Form der transkraniellen Gleichstromstimulation (30ˆmin pro Tag) sowie simultanes motorisches Training (Eine Stunde pro Tag). Eine Gruppe von fünf gesunden Probanden mit gleicher Altersverteilung diente als Kontrollgruppe. Für die Pyramidenbahn (PT) und für andere motorische Bahnen (aMF) wurden Die fraktionierte Anisotropie (FA), der scheinbare Diffusionskoeffizient (ADC) sowie radiale und axiale Diffusion bestimmt. Indices für hemisphärische Asymmetrie wurde für jeden Patienten/Probanden berechnet.

Ergebnisse:

Die Variabilität (ausgedrückt als Variationskoeffizient) war in allen untersuchten Parametern (FA, axiale Diffusion (λ_1), radiale Diffusion ($\lambda_{2,3}$) und ADC) am höchsten zwischen den Probanden. Inter-session Variabilität und Intra-session Variabilität hingegen, unterschieden sich nicht signifikant voneinander.

Ein Gruppenvergleich (Zweistichproben-t-Test) zwischen Patienten und der Kontrollgruppe zum Ausgangszeitpunkt ergab eine signifikant höhere Diffusivität in der vom Schlaganfall betroffenen Hemisphäre im Vergleich zu den gesunden Probanden. Die Verbesserung motorischer Fertigkeiten der Patienten war begleitet von signifikanten longitudinalen Veränderungen der Asymmetrieindices bezüglich ADC sowie radialer und axialer Diffusion aufgrund von verstärkter Diffusivität der aMF auf der betroffenen Seite nach therapeutischer Intervention. Die Änderungen in der Patientengruppe gingen in den folgenden Parametern über die normale Variabilität hinaus: FA, λ_1, ADC in der Pyramidenbahn sowie Trends in λ_1 und ADC in anderen motorischen Bahnen.

Konklusion:

Die Effekte der Schlaganfallrehabilitation konnten auf Bildgebungsebene in Form von Diffusivitätsasymmetrien der untersuchten Parameter gezeigt werden. Strukturelle Plastizität, begleitet von einer Verbesserung motorischer Fertigkeiten, spiegelte sich in den DTI-spezifischen Parametern wider. Dies könnte zukünftig genutzt werden, um das Ansprechen auf neurorehabilitative Therapien zu evaluieren. Allerdings muss die hohe Variabilität bei der Anwendung von DTI besonders bei der Untersuchung geringfügiger Änderungen beachtet werden.

12. References

1. Lindenberg, R., et al., *Structural integrity of corticospinal motor fibers predicts motor impairment in chronic stroke.* Neurology, 2010. **74**(4): p. 280-7.
2. Alexander, A.L., et al., *Diffusion tensor imaging of the brain.* Neurotherapeutics, 2007. **4**(3): p. 316-29.
3. Wiesendanger, M., *The pyramidal tract. Its structure and function. In: Tow AL, Luchei ES, eds. Motor coordination*. Handbook of behavionral neurobiology 5. 1981, New York: Plenum.
4. Nyberg-Hansen R, R.E., *Some comments on the pyramidal tract, with special reference to its individual variations in man.* Acta Neurol Scand, 1963. **39**: p. 1-30.
5. Glees, P. and J. Cole, *Ipsilateral representation in the cerebral cortex; its significance in relation to motor function.* Lancet, 1952. **1**(6720): p. 1191-2.
6. Noback, R., Srominger, L., Demarest, J., Ruggiero, A., *The human nervous system: structure and function*. 2005, Totawa: Humana Press.
7. Snell, R., *Clinical Neuroanatomy, 7th Edition*. 2010, Philadelphia: Lippincott Williams & Wilkins.
8. Moseley, M.E., et al., *Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system.* Radiology, 1990. **176**(2): p. 439-45.
9. Chenevert, T.L., J.A. Brunberg, and J.G. Pipe, *Anisotropic diffusion in human white matter: demonstration with MR techniques in vivo.* Radiology, 1990. **177**(2): p. 401-5.
10. Wiegell, M.R., H.B. Larsson, and V.J. Wedeen, *Fiber crossing in human brain depicted with diffusion tensor MR imaging.* Radiology, 2000. **217**(3): p. 897-903.
11. Mori, S. and J. Zhang, *Principles of diffusion tensor imaging and its applications to basic neuroscience research.* Neuron, 2006. **51**(5): p. 527-39.
12. Takagi, T., et al., *Visualization of peripheral nerve degeneration and regeneration: monitoring with diffusion tensor tractography.* Neuroimage, 2009. **44**(3): p. 884-92.
13. Wakana, S., et al., *Reproducibility of quantitative tractography methods applied to cerebral white matter.* Neuroimage, 2007. **36**(3): p. 630-44.

14. Ardolino, G., et al., *Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain.* J Physiol, 2005. **568**(Pt 2): p. 653-63.
15. Jefferys, J.G., *Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions.* Physiol Rev, 1995. **75**(4): p. 689-723.
16. Liebetanz, D., et al., *Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability.* Brain, 2002. **125**(Pt 10): p. 2238-47.
17. Nitsche, M.A., et al., *Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans.* J Physiol, 2003. **553**(Pt 1): p. 293-301.
18. Bindman, L.J., O.C. Lippold, and J.W. Redfearn, *The Action of Brief Polarizing Currents on the Cerebral Cortex of the Rat (1) during Current Flow and (2) in the Production of Long-Lasting after-Effects.* J Physiol, 1964. **172**: p. 369-82.
19. Schlaug, G., V. Renga, and D. Nair, *Transcranial direct current stimulation in stroke recovery.* Arch Neurol, 2008. **65**(12): p. 1571-6.
20. Webster, B.R., P.A. Celnik, and L.G. Cohen, *Noninvasive brain stimulation in stroke rehabilitation.* NeuroRx, 2006. **3**(4): p. 474-81.
21. Priori, A., *Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability.* Clin Neurophysiol, 2003. **114**(4): p. 589-95.
22. McCreery, D.B., et al., *Charge density and charge per phase as cofactors in neural injury induced by electrical stimulation.* IEEE Trans Biomed Eng, 1990. **37**(10): p. 996-1001.
23. Wagner, T., et al., *Transcranial direct current stimulation: a computer-based human model study.* Neuroimage, 2007. **35**(3): p. 1113-24.
24. Gluckman, B.J., et al., *Electric field suppression of epileptiform activity in hippocampal slices.* J Neurophysiol, 1996. **76**(6): p. 4202-5.
25. Purpura, D.P. and J.G. McMurtry, *Intracellular Activities and Evoked Potential Changes during Polarization of Motor Cortex.* J Neurophysiol, 1965. **28**: p. 166-85.

26. Lefaucheur, J.P., *Methods of therapeutic cortical stimulation.* Neurophysiol Clin, 2009. **39**(1): p. 1-14.
27. Konishi, J., et al., *MR tractography for the evaluation of functional recovery from lenticulostriate infarcts.* Neurology, 2005. **64**(1): p. 108-13.
28. Kunimatsu, A., et al., *Three-dimensional white matter tractography by diffusion tensor imaging in ischaemic stroke involving the corticospinal tract.* Neuroradiology, 2003. **45**(8): p. 532-5.
29. Lee, J.S., et al., *Fiber tracking by diffusion tensor imaging in corticospinal tract stroke: Topographical correlation with clinical symptoms.* Neuroimage, 2005. **26**(3): p. 771-6.
30. Yamada, K., et al., *Stroke patients' evolving symptoms assessed by tractography.* J Magn Reson Imaging, 2004. **20**(6): p. 923-9.
31. Nelles, M., et al., *Diffusion tensor pyramidal tractography in patients with anterior choroidal artery infarcts.* AJNR Am J Neuroradiol, 2008. **29**(3): p. 488-93.
32. Kang, D.W., et al., *Diffusion-weighted imaging in Wallerian degeneration.* J Neurol Sci, 2000. **178**(2): p. 167-9.
33. Lindberg, P.G., et al., *Wallerian degeneration of the corticofugal tracts in chronic stroke: a pilot study relating diffusion tensor imaging, transcranial magnetic stimulation, and hand function.* Neurorehabil Neural Repair, 2007. **21**(6): p. 551-60.
34. Thomalla, G., et al., *Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke.* Neuroimage, 2004. **22**(4): p. 1767-74.
35. Werring, D.J., et al., *Diffusion tensor imaging can detect and quantify corticospinal tract degeneration after stroke.* J Neurol Neurosurg Psychiatry, 2000. **69**(2): p. 269-72.
36. Yu, C., et al., *A longitudinal diffusion tensor imaging study on Wallerian degeneration of corticospinal tract after motor pathway stroke.* Neuroimage, 2009. **47**(2): p. 451-8.
37. Heiervang, E., et al., *Between session reproducibility and between subject variability of diffusion MR and tractography measures.* Neuroimage, 2006. **33**(3): p. 867-77.

38. Reich, D.S., et al., *Quantitative characterization of the corticospinal tract at 3T.* AJNR Am J Neuroradiol, 2006. **27**(10): p. 2168-78.
39. Schaechter, J.D. and K.L. Perdue, *Enhanced cortical activation in the contralesional hemisphere of chronic stroke patients in response to motor skill challenge.* Cereb Cortex, 2008. **18**(3): p. 638-47.
40. Schaechter, J.D., K.L. Perdue, and R. Wang, *Structural damage to the corticospinal tract correlates with bilateral sensorimotor cortex reorganization in stroke patients.* Neuroimage, 2008. **39**(3): p. 1370-82.
41. Imfeld, A., et al., *White matter plasticity in the corticospinal tract of musicians: a diffusion tensor imaging study.* Neuroimage, 2009. **46**(3): p. 600-7.
42. Gladstone, D.J., C.J. Danells, and S.E. Black, *The fugl-meyer assessment of motor recovery after stroke: a critical review of its measurement properties.* Neurorehabil Neural Repair, 2002. **16**(3): p. 232-40.
43. Fugl-Meyer, A.R., et al., *The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance.* Scand J Rehabil Med, 1975. **7**(1): p. 13-31.
44. Thomas, B., et al., *Quantitative diffusion tensor imaging in cerebral palsy due to periventricular white matter injury.* Brain, 2005. **128**(Pt 11): p. 2562-77.
45. Weinstein, D.M., G.L. Kindlmann, and E.C. Lundberg, *Tensorlines: Advection-diffusion based propagation through diffusion tensor fields. .* IEEE Visualization, 1999: p. 249-254.
46. Talairach J, T.P., *Co-planar stereotaxic atlas of the human brain*. 1988, New York, Stuttgart: Thieme.
47. Cercignani, M., et al., *Inter-sequence and inter-imaging unit variability of diffusion tensor MR imaging histogram-derived metrics of the brain in healthy volunteers.* AJNR Am J Neuroradiol, 2003. **24**(4): p. 638-43.
48. Landis, J.R. and G.G. Koch, *The measurement of observer agreement for categorical data.* Biometrics, 1977. **33**(1): p. 159-74.
49. Bonekamp, D., et al., *Diffusion tensor imaging in children and adolescents: reproducibility, hemispheric, and age-related differences.* Neuroimage, 2007. **34**(2): p. 733-42.
50. Ciccarelli, O., et al., *From diffusion tractography to quantitative white matter tract measures: a reproducibility study.* Neuroimage, 2003. **18**(2): p. 348-59.

51. Hummel, F. and L.G. Cohen, *Improvement of motor function with noninvasive cortical stimulation in a patient with chronic stroke.* Neurorehabil Neural Repair, 2005. **19**(1): p. 14-9.
52. Hesse, S., et al., *Combined transcranial direct current stimulation and robot-assisted arm training in subacute stroke patients: a pilot study.* Restor Neurol Neurosci, 2007. **25**(1): p. 9-15.
53. Uy, J. and M.C. Ridding, *Increased cortical excitability induced by transcranial DC and peripheral nerve stimulation.* J Neurosci Methods, 2003. **127**(2): p. 193-7.
54. Loubinoux, I., et al., *Correlation between cerebral reorganization and motor recovery after subcortical infarcts.* Neuroimage, 2003. **20**(4): p. 2166-80.
55. Nair, D.G., et al., *Imaging correlates of motor recovery from cerebral infarction and their physiological significance in well-recovered patients.* Neuroimage, 2007. **34**(1): p. 253-63.
56. Chollet, F., et al., *The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography.* Ann Neurol, 1991. **29**(1): p. 63-71.
57. Cramer, S.C., et al., *A functional MRI study of subjects recovered from hemiparetic stroke.* Stroke, 1997. **28**(12): p. 2518-27.
58. Nelles, G., et al., *Reorganization of sensory and motor systems in hemiplegic stroke patients. A positron emission tomography study.* Stroke, 1999. **30**(8): p. 1510-6.
59. Strens, L.H., et al., *The ipsilateral human motor cortex can functionally compensate for acute contralateral motor cortex dysfunction.* Curr Biol, 2003. **13**(14): p. 1201-5.
60. Schaechter, J.D., et al., *Microstructural status of ipsilesional and contralesional corticospinal tract correlates with motor skill in chronic stroke patients.* Hum Brain Mapp, 2009. **30**(11): p. 3461-74.
61. Chen, Z., et al., *Evaluating ischemic stroke with diffusion tensor imaging.* Neurol Res, 2008. **30**(7): p. 720-6.
62. Kerschensteiner, M., et al., *In vivo imaging of axonal degeneration and regeneration in the injured spinal cord.* Nat Med, 2005. **11**(5): p. 572-7.

63. Ludwin, S.K., *Oligodendrocyte survival in Wallerian degeneration.* Acta Neuropathol, 1990. **80**(2): p. 184-91.
64. Carmichael, S.T., *Cellular and molecular mechanisms of neural repair after stroke: making waves.* Ann Neurol, 2006. **59**(5): p. 735-42.
65. Nudo, R.J., et al., *Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct.* Science, 1996. **272**(5269): p. 1791-4.
66. Nudo, R.J. and G.W. Milliken, *Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys.* J Neurophysiol, 1996. **75**(5): p. 2144-9.
67. Jaillard, A., et al., *Vicarious function within the human primary motor cortex? A longitudinal fMRI stroke study.* Brain, 2005. **128**(Pt 5): p. 1122-38.
68. Miyai, I., et al., *Middle cerebral artery stroke that includes the premotor cortex reduces mobility outcome.* Stroke, 1999. **30**(7): p. 1380-3.
69. Dancause, N., et al., *Extensive cortical rewiring after brain injury.* J Neurosci, 2005. **25**(44): p. 10167-79.
70. Beaulieu, C., *The basis of anisotropic water diffusion in the nervous system - a technical review.* NMR Biomed, 2002. **15**(7-8): p. 435-55.
71. Fries, W., A. Danek, and T.N. Witt, *Motor responses after transcranial electrical stimulation of cerebral hemispheres with a degenerated pyramidal tract.* Ann Neurol, 1991. **29**(6): p. 646-50.
72. Lang, C.E. and M.H. Schieber, *Human finger independence: limitations due to passive mechanical coupling versus active neuromuscular control.* J Neurophysiol, 2004. **92**(5): p. 2802-10.
73. Lawrence, D.G. and H.G. Kuypers, *The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways.* Brain, 1968. **91**(1): p. 15-36.
74. Schmahmann, J.D., R. Ko, and J. MacMore, *The human basis pontis: motor syndromes and topographic organization.* Brain, 2004. **127**(Pt 6): p. 1269-91.

Publications

Peer-reviewed articles

Lindenberg R, Renga V, Zhu LL, **Betzler F**, Alsop D, Schlaug G, *Structural integrity of corticospinal motor fibers predicts motor impairment in chronic stroke.* Neurology 2010; 74(4): p. 280-7

Peer-reviewed abstracts

Schlaug G, **Betzler F**, Zhu LL, Lindenberg R, *Plastic Changes in Corticospinal Motor Tracts Are Related to Motor Improvement in Chronic Stroke Patients*. Stroke 2010; 41: p. S210

Betzler F, Lindenberg R, Zhu LL, Schlaug G. *Differentiating normal variability from structural plasticity: DTI studies in stroke patients undergoing therapy.* NeuroImage 2009; 47: p. S74

Lindenberg R, Renga V, Zhu LL, **Betzler F**, Nair D, Schlaug G. *Relating motor impairment to structural integrity of corticospinal motor fibers using diffusion tensor imaging*. NeuroImage 2009; 47: p. S94

Betzler F, Schlabs T, Wagenseil B, Gewies M, Abels W, Schulz J, Kowoll R, Gunga H.C., *Can facilitation increase the H-reflex in micro-g?* ELGRA News 2007; 25: p. 162-3

Selbständigkeitserklärung

Ich, Felix Betzler, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: „Structural changes in motor tracts of chronic stroke patients associated with experimental rehabilitation: a DTI study“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.