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Cortical representation of motor functions in individuals with rotationplasty

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Abbreviations

BET	Brain extraction tool
BOLD	Blood oxygen level dependent
BSC	BOLD signal change
fMRI	Functional magnetic resonance imaging
M1	Primary motor cortex
MNI	Montreal Neurologic Institute
MRI	Magnetic resonance imaging
PLP	Phantom limb pain
ROI	Region of interest
RP	Rotationplasty
S1	Primary somatosensory cortex
TMS	Transcranial magnetic stimulation

1. Introduction

Cortical reorganization describes the process of adaption of an established cortical network (von Bernhardi et al., 2017) which can occur as a consequence of task-induced learning (Jenkins et al., 1990), or after disruption of the central or peripheral nervous system by stroke (Dimyan & Cohen, 2011), or peripheral nerve lesion (Navarro et al., 2007). Adaptive cortical reorganization is therefore crucial for the acquisition of new skills as well as for recovery after injury of the nervous system. Cortical reorganization can however also be maladaptive, leading to abnormal sensory phenomena, such as phantom pain (Flor et al., 1995), or chronic back pain (Hotz-Boendermaker et al., 2016).

The relationship between cortical reorganization and sensory phenomena has been widely studied, but the findings reported in the literature are mixed. In 1995, Flor et al. showed a close association between the extent of cortical map changes in the primary somatosensory cortex (S1) and the severity of phantom limb pain (PLP) in upper limb amputees. A larger shift of the mouth representation, which is adjacent to the hand area on the cortical map (Penfield & Rasmussen, 1950), into the zone formerly representing the arm correlated with more pronounced phantom limb pain. Several studies reproduced these findings in the S1 cortex (Grüsser et al., 2001; Flor et al., 1998) as well as in the motor cortex (Wu & Kaas, 1999; Schwenkreis, 2001; Mercier & Léonard, 2011), supporting this maladaptive plasticity model (Karl et al., 2001; Karl et al., 2004; Lotze et al., 2001). Besides phantom pain, additional factors might contribute to cortical reorganization. For example, poorer voluntary motor control of the phantom limb and higher levels of pain in the phantom limb have been shown to be drivers of cortical reorganization of the lip and proximal upper-limb topology (Raffin et al., 2016).

An alternative view on cortical reorganization associated with a missing limb was suggested by Makin et al. (2013). In this study, the authors did not report a shift of adjacent cortical areas to the former hand area but showed that abnormal sensory phenomena might arise from increased neural activity in the former hand area. Although seemingly contradictory, both findings might well coexist if one considers methodological differences between these studies (Andoh et al., 2020). Discrepancies between these studies may arise from the type of tasks being performed, e.g., execution or imagery of phantom movements or the involvement of stump muscles. An additional factor of variability could be related to the definition of regions of interest (ROIs), i.e., based on an individual cortical peak of activation or based on a group average (Andoh et al., 2020). Moreover, discrepancies between studies might also arise if one examines the cortical area of the missing limb versus adjacent cortical areas to the missing limb (e.g., the mouth in upper limb amputees) (Flor et al., 1995; Makin et al., 2013; Raffin et al., 2016; Moseley, 2006). All approaches might provide complementary information about functional changes occurring after an amputation but they do, however, suffer from a special challenge as they investigate a body part, which is absent.

An interesting group to consider for assessing the cortical representation of a partly missing limb is a group of patients who underwent a rotationplasty surgery. Such patients have undergone a rare surgical intervention in which a diseased part of the proximal leg including the knee is removed while the neurovascular bundle is spared. The distal part of the leg is then re-attached to the thigh by a rotation through 180°. The ankle now acts as a knee joint while the foot is facing backward (Bernthal et al., 2014). Thus, these subjects provide the intriguing combination of an intact central and peripheral nervous system while the body scheme (defined as the internal awareness of the body and the relationship of body parts to one another) is significantly altered. In the present thesis, cortical reorganization in this unique group of patients is investigated. In contrast to amputees, these patients allow for a direct mapping of the cortical representation of motor and sensory functions without using surrogate tasks, such as motor imagery (defined as the mental execution of a movement without an overt movement or muscle activation), phantom limb movement or sensory stimulation of adjacent areas. Moreover, patients with rotationplasty generally do not suffer from PLP (Frassica et al., 1988).

Cortical representation and reorganization after rotationplasty surgery has so far been studied very rarely. Curtze et al. (2010) investigated the mental rotation of the feet in one subject with rotationplasty in comparison to a cohort of lower limb amputees and of controls. The patient with rotationplasty as well as the amputees did not differ in their ability to identify illustrations of their affected limb in comparison to their contralateral limb while the control group showed clear laterality effects. This finding could result from the use of an innate, prototypical representation of the body by patients with rotationplasty as well as amputees rather than using motor imagery to solve the task like control subjects.

Cortical reorganization has been studied via transcranial magnetic stimulation (TMS) in a cohort of three patients (Tesio et al., 2014). This study showed hemispheric asymmetries with increased cortical excitability and spatial overrepresentation of the unrotated soleus and vastus medialis compared to the rotated soleus and the muscles of healthy controls. However, the spatial resolution of TMS as well as the large overlap of the cortical representations of the soleus and vastus medialis muscles did not allow for a detailed mapping of the locations of the cortical representations of these muscles. Thus, questions regarding a possible shift of the cortical representation of the rotated part of the lower limb after rotationplasty surgery remains unsolved.

We therefore aimed to investigate:

- If the motor cortical representation of the rotated limb undergoes cortical reorganization.
- If neural activity in the motor cortical representation of the rotated limb is decreased in comparison to the intact limb.

The present thesis describes the investigation of the motor cortex using functional magnetic resonance imaging (fMRI) in a cohort of patients with a rotationplasty. The findings from this work should provide a deeper insight into the cortical organization of the lower limbs and a better understanding of the mechanisms of cortical reorganization.
The understanding of processes of adaptive cortical reorganization leading to (partial) recovery as well as processes of maladaptive cortical reorganization leading to impaired functional recovery or chronic pain is not only of academic interest but plays a role in the rehabilitation of patients. For instance, the training of upper limb amputees with a brain–machine interface using real-time magnetoencephalography signals to reconstruct the hand movements of the missing hand with a robotic hand has been shown to reduce PLP if the training was designed to dissociate the prosthetic and phantom hand while a training designed for functional restoration of the phantom hand intensified the pain (Yanagisawa et al., 2016). Moreover, the determination of brain regions playing a role in recovery may help to optimize fMRI biofeedback interventional trials and the measurement of plasticity reserves via fMRI appears to be a promising predictor of clinical outcome (De Giglio et al., 2018).

1.1 Rotationplasty

Rotationplasty is a limb-sparing reconstructive option for patients in need of a resection of a proximal portion of the lower limb without involvement of its distal portion. Thus, it constitutes an alternative to amputation and endoprothetic surgery. The procedure involves the resection of a proximal part of the lower limb while the distal part as well as the neurovascular bundle are spared, rotated by 180° and grafted to the proximal part of the leg (fig.1). The rotation is necessary as the ankle flexes in the opposite direction compared to the knee. After fitting of a modified below-knee-prothesis the ankle fulfills the function of the knee, allowing for a prothesis with only one joint instead of two as needed after above-knee-amputation.

This technique was first introduced by Borggreve (Borggreve, 1930) in a patient with leg shortening due to tuberculosis and popularized for the management of proximal femoral focal deficiency by Van Nes in 1950 (Van Nes, 1950). In 1981 Salzer et al. first reported using rotationplasty as a reconstruction in the context of oncologic resections. Today, rotationplasty is most commonly used for lower-limb reconstruction in skeletally immature patients suffering from osteosarcoma in the distal femur or proximal tibia.

In a large, multi-institutional study functional results of rotationplasty are reported to be favorable in comparison to alternative limb-sparing procedures or amputation (Ginsberg et al., 2007). Furthermore, patients who have undergone rotationplasty are often returning to high-impact sports, such as skiing or running (Bernthal et al. 2014). Oncologic outcome depends on tumor stage and surgical margins while the reconstructive methods appear to be equivalent (Gupta et al., 2012).

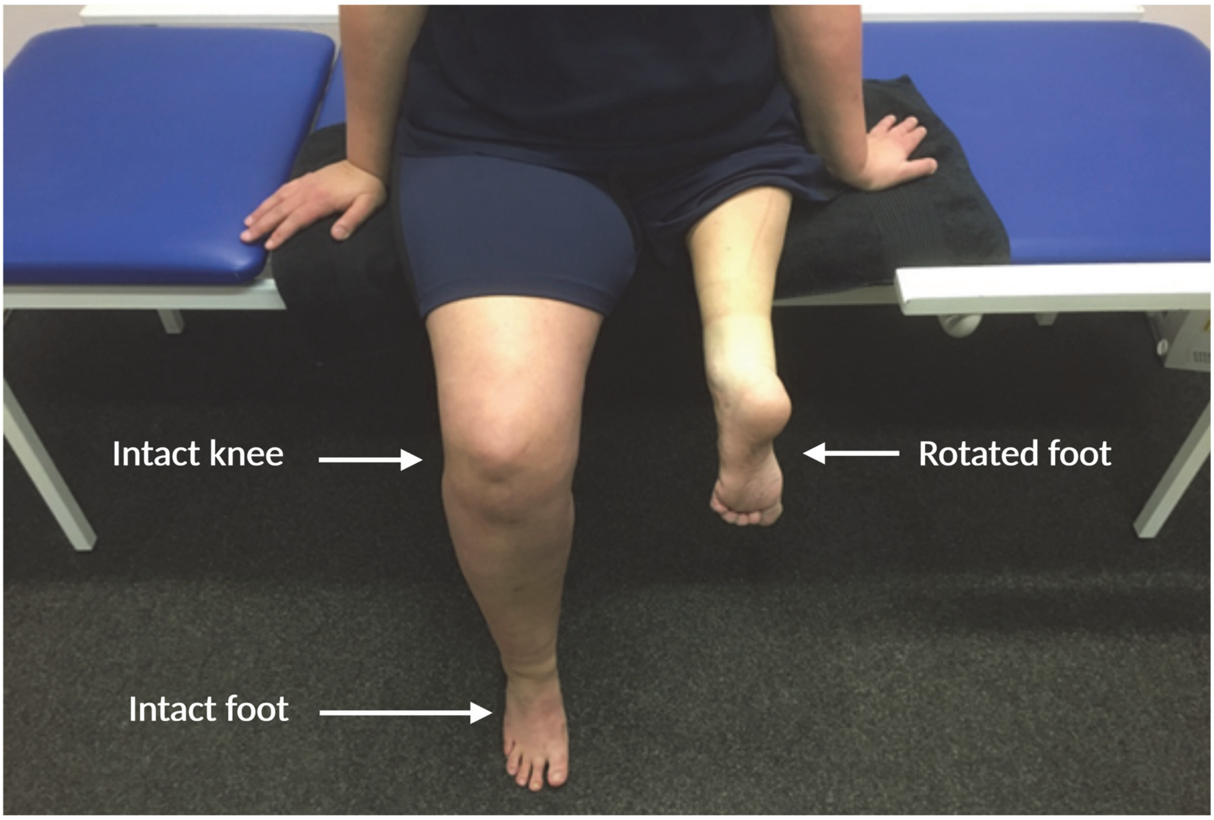

Figure 1: Patient with a rotationplasty surgery from our cohort. Positions of the intact knee, intact foot and rotated foot are indicated. These terms are used throughout the present thesis.

Several modifications of the rotationplasty exist. Type A I rotationplasty involves the resection of the distal femur and the knee while type A II involves the resection of the knee and proximal tibia. In type B I and B II rotationplasties the proximal femur is resected while the knee is spared and functions as a hip joint while the ankle serves as a knee joint. Type B II rotationplasty includes the resection of the acetabulum due to hip involvement. In type B III rotationplasty the entire femur is resected. The reconstruction of the hip is either performed by forming a pseudofemoral head out of the tibial plateau or by insertion of a hip hemiarthroplasty implant into the rotated tibia.

As all subjects in this study underwent a type A I rotationplasty, the further focus will lie on this technique. In this operation the rectus femoris origin is connected to the gastrocnemius and the hamstrings origins are sutured to the anterior aspect of the proximal tibia (fig. 2). Thus, the ankle, now functioning as the knee, is still driven by normal ankle-driving muscles and its respective nerves.

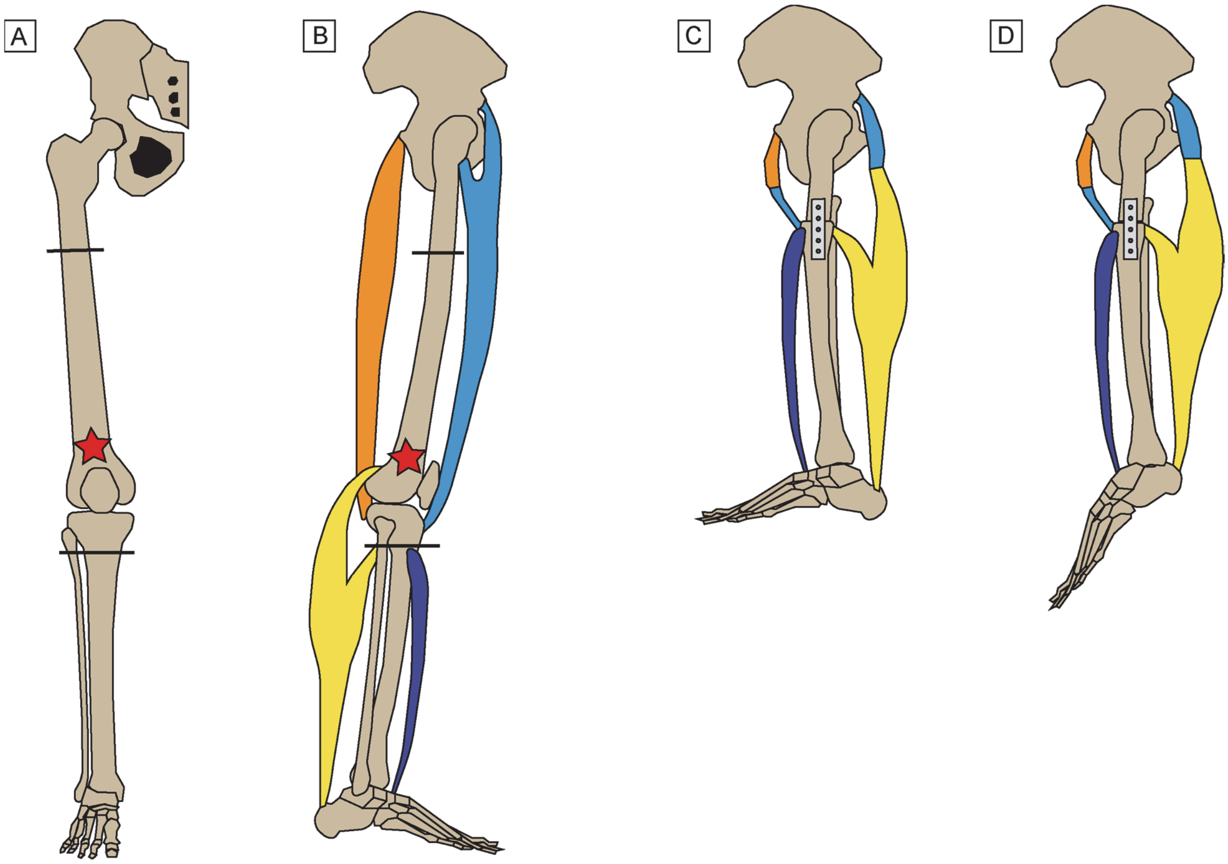

Figure 2: Drawings representing Type A I rotationplasty for a distal femur tumor. (A) Anterior and (B) lateral views of a distal femur tumor (red star) with normal muscle groups noted in the lateral view. Black lines represent the approximate bone cut levels of a Winkelmann A I resection. (C) Lateral bone and soft tissue view of the rotationplasty reconstruction in neutral position showing the rectus femoris origin sutured to the gastrocnemicus, and the hamstrings origins sutured to the proximal tibia. (D) Lateral bone and soft tissue view of the rotationplasty reconstruction in extension of the rotated foot, mediated by contraction of the gastrocnemicus and soleus. The ankle (now the knee) is still driven by normal ankle-driving muscles. (Key: light blue = quadriceps, orange = hamstrings, yellow = gastrocnemicus and soleus, dark blue = tibialis anterior).
Figure adapted from Bernthal et al. (2014) with permission of Elsevier Science & Technology Journals.

1.2 Cortical organization of the motor and somatosensory cortex of the lower limb

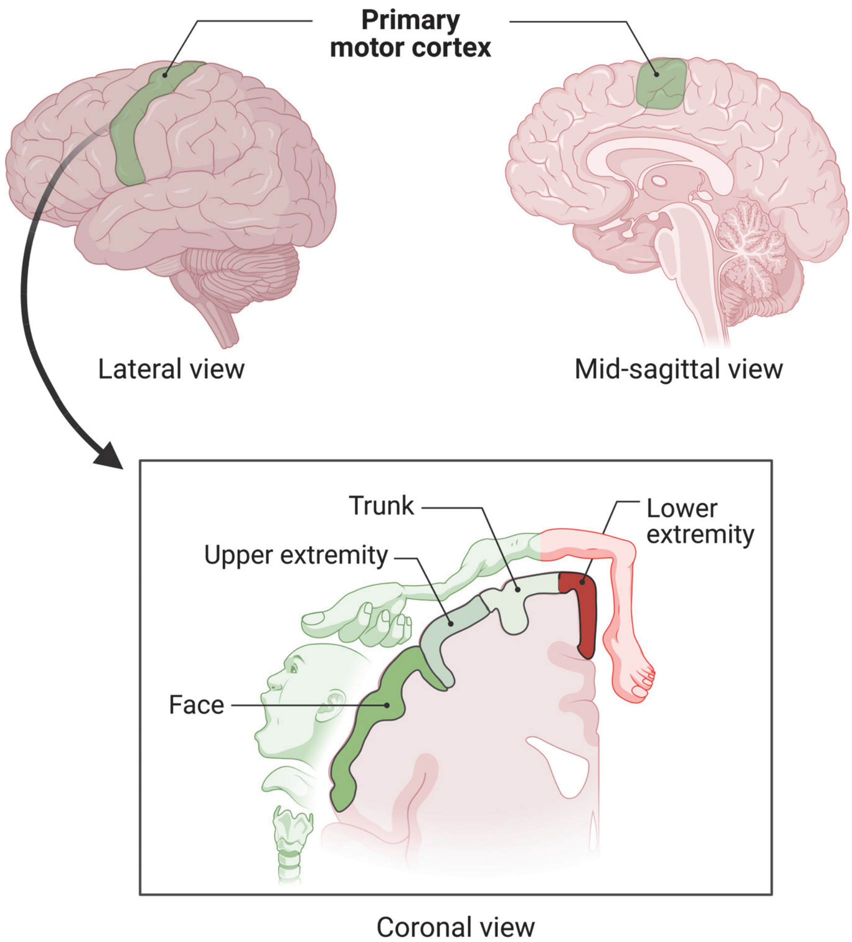

Figure 3: Topographic map of the primary motor cortex, modified from Penfield and Rasmussen's homunculus (Penfield. & Rasmussen, 1950). The cortical representation of the leg is highlighted in red. Adapted from "Topographic Map of the Motor Cortex", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

The somatotopic cortical organization of the body, known as the homunculus (fig. 3), has first been described by Penfield and Rasmussen (Penfield. & Rasmussen, 1950). In order to draw this cortical map, the authors used electrostimulation of the brain surface. Upon a positive response of the patient, small squares of paper were placed on the brain surface and the response of the patient was noted. In addition, the positions of the paper were sketched on a brain chart, showing a 3D view of the right hemisphere (Penfield & Boldrey, 1937). The lower extremity was found at the mesial surface of the precentral gyrus for the motor homunculus and of the postcentral gyrus for the sensory homunculus respectively with the proximal leg located most laterally and the foot located most medially. The motor homunculus shares the main features with the somatosensory homunculus with only slight variations of the relative area taken up by the individual body parts. Noteworthy, the leg and foot areas are difficult to access because they are located buried in the border of the paracentral lobe and in proximity of large blood vessels. These regions are therefore difficult to stimulate, thus yielding a less detailed resolution than neighboring brain regions. Since the discovery of the somatotopy of body representation by Penfield and Rasmussen, the homunculus has been reproduced and updated by implementation of modern technology that allows for a noninvasive investigation of the cortical organization. Kapreli et al. (2007) used fMRI to map joint movements of the lower extremity. Participants were instructed to flex and extend either the knee, the ankle or the toes or to perform a finger-to-thumb opposition. The clearest shift in alignment of the mean center of mass in the contralateral sensorimotor cortex was found in the medial to lateral direction, thus toe, ankle, knee and finger activation shifting consistently from medial to lateral locations. The authors found a large overlap of the representations in the primary motor cortex during the movement of the lower limb joints. Nevertheless, not only the fingers were found to present a statistically significant center of mass of cortical activation, but also within the lower limb a statistically significant separation between knee and ankle as well as between knee and toes was evident. The overlap between the cortical representation of lower limb joints could in part be explained by contraction of bi-articular muscles of the leg. As these muscles can move two different joints, the movement can either take place at the origin or the insertion of the muscle, depending on which joint is fixed. For example, the gastrocnemius muscle can either flex the knee or flex the ankle. Further examples for bi-articular muscles of the leg are the rectus femoris and the biceps femoris. In addition, multi-articular muscles like the extensor digitorum longus, tibialis anterior, peroneus brevis and longus, tibialis posterior and flexor hallucis longus complicate the isolation of the mechanical output in one joint, even if there is a restriction of the range of motion. In order to perform an isolated joint movement, a certain degree of simultaneous control of neighboring joints being stabilized during the target motion task is required (Hager-Ross & Schieber, 2000; Humphrey & Reed, 1983; Schieber, 1991). Moreover, the sensorimotor topographic overlap could be caused by the provoked afferent input due to the joint movement as the movement of an isolated joint recruits sensory input from different mechanoreceptors such as tendons, ligaments, capsule or muscles of the same joint or of neighboring joints (Hogervorst & Brand, 1998; Proske et al., 2000). This is especially true for multi-joint connections via bi-articular muscles and fascia.

As Penfield and Rasmussen's homunculus was obtained by direct and focal cortical stimulation, several factors of the physiological connections and circuitry of the motor cortex were not taken into account (Schieber, 2001). Such factors include the activation of differing motor outputs by a single cortical site known as divergent output. Analogously, convergence refers to one motor output activated by two or more separate cortical motor sites. Additional factors such as horizontal connections, a widely distributed activity, partial inactivity and the plasticity of the brain explain why there is no direct processing relationship between the motor cortex and the muscles.

A somatotopic organization of the leg and foot representation in the somatosensory cortex has been confirmed via ultra-high field MRI (7 T) by mapping six cortical representations of the lower limb from hip to toes (Akselrod et al., 2017). In all participants, lower limb representations were located in the superior part of the postcentral gyrus in the hemisphere contralateral to the stimulated foot. The hip, thigh and calf representations were located most laterally and appeared in an orderly manner moving from lateral to medial positions followed by the foot representation. However, there was no consistent ordering of foot representations (big toe, small toe, heel) along the somatosensory cortex contrary to the fingers (Janko et al., 2022). Moreover, substantial inter-subject variability regarding the exact sequence and location of each of the mapped representations within S1 was observed. Such strong inter-individual variability was already reported for non-human primates (Merzenich et al., 1978), showing that a given body region is not at the same position in different individuals. Akselrod et al. (2017) therefore emphasized the importance of studies focusing on single subject analysis, as small cortical representations might not overlap across individuals, suggesting that generalizations based on group analysis should be regarded with caution.

Evoked magnetic fields and source analysis allow for a noninvasive but precise mapping of the leg representation. With this technique, Dietrich et al. (2017) showed that the localization of the leg in the somatosensory cortex follows the dermatomal organization of spinal nerves instead of the typical map of neighboring body parts as shown in Penfield and Rasmussen's somatosensory homunculus. Thus, the cortical representation of the back of the thigh is located inferior to the foot's representation in the somatosensory cortex while the front of the thigh is located laterally to the foot's representation.

1.3 Advantages and challenges of MRI for the investigation of cortical reorganization

Since its introduction in the clinic in the 1980s, magnetic resonance imaging (MRI) plays an indispensable role in diagnostic medicine. Furthermore, since the 1990s several MRI-techniques, but in particular functional magnetic resonance imaging (fMRI), have been established as a standard in basic research neuroscience. A recent database (PubMed) query with the keywords “functional magnetic resonance imaging” or “functional MRI” or “fMRI” returned over 600,000 peer reviewed articles.

In contrast to Penfield and Rasmussen’s approach to define the homunculus by direct electrical cortical stimulation, fMRI allows for a noninvasive method to investigate the human cortex with a relatively high spatiotemporal resolution. Moreover, fMRI is able to demonstrate entire neuronal networks involved in a specific task.

A particular strength of fMRI lies in its high spatial resolution allowing for the investigation of somatotopy of the cortex. The typical 3 T fMRI pixel size is 3–4 mm (Glover, 2011) but with the help of higher field magnets (7T) a pixel size of 500 microns or less can be achieved (Shmuel et al., 2007). Despite an overlap in the representation of neighboring body parts in the primary somatosensory and motor cortex, fMRI enables the mapping of within-limb somatotopy which has been shown in multiple publications (Kapreli et al., 2007; Plow et al., 2010; Akselrod et al., 2017; Willoughby et al., 2021).

Neural plasticity refers to the ability of established neural networks within the brain to undergo alterations or modifications. With the help of structural MRI and fMRI, correlates of neural plasticity such as changes in cortical thickness, intensity of activation, localization of maximal neural activity or the area of excitation can be determined and quantified. Examples of such changes are the enlargement of grey matter in the hippocampus of London taxi drivers adapting to the task of navigation in a metropole (Maguire et al., 2000), cortical reactivation after sensory loss in the hand of primates (Qi et al., 2021) or the shift of the cortical mouth representation into the area of an amputated arm in patients suffering from PLP (Flor et al., 1995).

FMRI is based on the measurement of hemodynamic changes. Thus, its temporal resolution is limited by the hemodynamic response time. Following a lag of 2-6 seconds the blood oxygenation level dependent (BOLD) response reaches a peak around 5–6 seconds after the onset of a neural stimulus. This response is considerably slower than the underlying neural process. Nevertheless, by choosing an appropriate study design (most often block design) and sophisticated analysis methods, temporal inferences in the 100ms resolution range can be achieved (Glover, 2011). A further challenge of fMRI is the measurement of the hemodynamic response as a surrogate signal for neural activity. For this reason, fMRI cannot distinguish between function-specific processing and neuromodulation and can potentially confuse excitation and inhibition (Logothetis, 2008).

2. Aims and goals

We aimed to investigate if the cortical representation of motor functions in the lower limb in patients with rotationplasty is preserved or if it has undergone reorganization. For this purpose, we carried out fMRI during motor tasks in a sample of ten patients with rotationplasty and in ten matched controls. We assessed differences in cortical representation of the motor functions between the location of the intact and the rotated limbs. In addition, we assessed differences in task-related activity in the motor cortex between rotationplasty patients and controls.

The main hypotheses in the current thesis are as follows:

Hypothesis 1: In the rotationplasty group, we expected decreased neural activity during movements of the rotated foot compared with movements of the intact foot. In addition, we expected the location of the rotated foot in the rotationplasty group to be at the level of the knee representation of the contralateral hemisphere.

Hypothesis 2: The rotationplasty group was expected to show decreased neural activity during movements of the rotated foot compared with movements of the foot in controls. In addition, we expected the location of the rotated foot in the rotationplasty group to be at the level of the knee representation in the control group.

3. Materials and methods

3.1 Participants

Fifteen participants with an A I rotationplasty were initially recruited for this study, four of whom were not eligible due to MRI-incompatible metal implants. One participant who was recruited before planned rotationplasty surgery dropped out of the study as she received an amputation instead. Ten subjects with an A I rotationplasty (five females and five males) aged between 18 and 54 years (mean±SD 35.9±10.7 years) participated in this study. Five subjects had a right rotationplasty. One subject was lefthanded. On average, the rotationplasty operation was performed 20.2±6.9 years prior to the study.

A structured interview about the characteristics and reason for the rotationplasty surgery, prothesis use and phantom phenomena was carried out. In addition, participants completed the German version of the West Haven-Yale Multidimensional Pain Inventory (MPI) (Flor et al., 1990; Kerns et al., 1985) to assess PLP. PLP intensity was defined by the MPI pain intensity subscale.

Ten healthy controls matched by age (mean±SD 36.2±9.3 years), sex and handedness were recruited from the general population. Demographic and clinical characteristics of our cohort are summarized in table 1.

All participants were informed about the nature of the experimental procedures and all subjects gave written informed consent prior to the study. The study was approved by the Ethics Committee of the Medical Faculty Mannheim, Heidelberg University. The study protocol adhered to the Declaration of Helsinki.

Table 1: Demographic and clinical characteristics of our cohort. M = male, F = female, RP = rotationplasty, PLP = phantom limb pain, R = right, L = left.

	ID	Sex	Age at participation [years]	Side of RP	Age at RP operation [years]	Reasons for RP	Prothesis use	PLP (scale 0-6)
Rotationplasty	RP01	M	38	R	18	tumor	All day	0
	RP02	M	26	R	13	tumor	All day	0
	RP03	F	18	R	5	tumor	All day	0
	RP04	M	32	L	14	tumor	All day	0
	RP05	M	50	R	20	tumor	All day	0
	RP06	M	54	R	23	tumor	Almost daily for a few hours/day	2
	RP07	F	40	L	29	tumor	All day	0
	RP08	F	34	L	14	tumor	All day	0
	RP09	F	38	L	16	tumor	All day	0
	RP10	F	29	L	5	tumor	All day	0
Controls	C01	M	38					
	C02	M	25					
	C03	F	25					
	C04	M	32					
	C05	M	50					
	C06	M	54					
	C07	F	38					
	C08	F	38					
	C09	F	34					
	C10	F	28					

3.2 MR scanning procedure

3.2.1 fMRI paradigm

All participants were scanned lying down in a supine position inside the MR scanner with their head immobilized and their eyes open. The motor task consisted of an extension and flexion of three body sites for participants with a rotationplasty (knee, intact foot and rotated foot) and four body sites for the control group (left and right knee, left and right foot). The order of movement was counterbalanced across the participants. To facilitate the movement of the knee and rotationplasty, a rigid foam block was placed under the knee/rotated foot to achieve an angle of about 45° of the knee/rotated foot. The subjects were trained to fully extend (0°) the knee/rotated foot and subsequently flex it again to reach the resting position. In order to perform an extension and flexion of the intact foot and the feet of control subjects, a rigid foam block was placed under the calf so that full dorsiflexion and plantar extension of the foot could be executed. Prior to the experiment all subjects were trained inside the scanner to perform the correct degree of movement with as little as possible movement of the head or other body parts. All participants practiced the correct movement until a steady and stable movement of the respective joint was achieved. The correct performance was controlled visually by the examiners during the scanning procedure. For each body site, a block design was used for the experiment consisting of ten movement blocks interleaved with ten rest periods (baselines) lasting each for 20s lasting in total 10.47 min. In order to pace the movements at a rate of 0.5Hz, the subjects received a visual cue. Either a blue rhombus or a yellow square was presented to the participants on a screen at the end of the MRI scanner via a 45° oriented mirror placed above the eyes while they were lying down inside the MR scanner. Participants were instructed to perform flexion/extension of the knee, rotated foot or intact foot during the rhombus presentation and to rest during the square presentation. The experimental procedure is visualized for knee flexion/extension in figure 4.

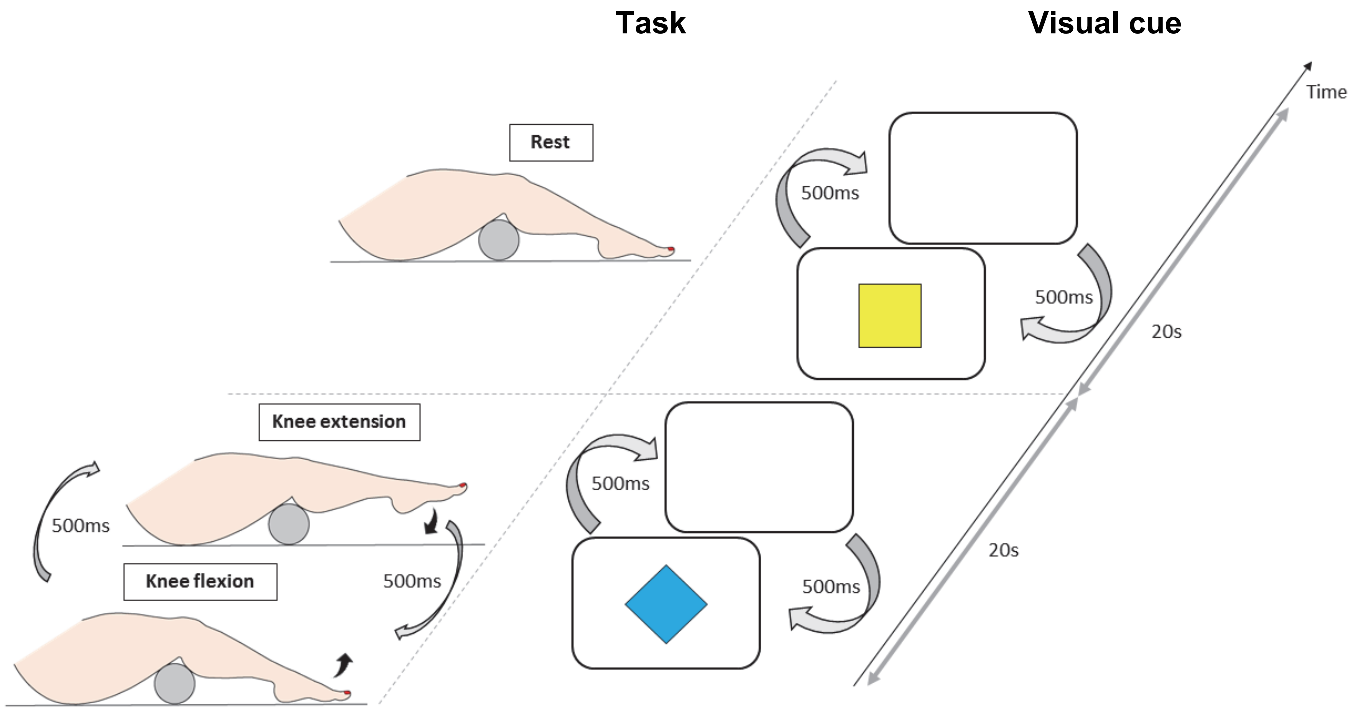

Figure 4: Experimental procedure for knee flexion and extension.

3.2.2 MRI data parameters

Magnetic Resonance Imaging (MRI) data were acquired on a 3 Tesla whole body scanner (SIEMENS TRIO) with a 32-channel head coil. A structural T1-weighted 3D scan was acquired using a gradient echo sequence (TR/TE = 2300/2.98, 192 slices, matrix 256 x 256) with a 1mm isotropic resolution (duration 6 min). Functional MRI (fMRI) was acquired using a T_2*-weighted sequence (40 slices, slice thickness= 2 mm, TR/TE= 3000/27, flip angle 90°, matrix size 192 x 192, FOV= 220 x 220 mm, in-plane resolution 1.5 x 1.5 mm).

3.2.3 MR data preprocessing

All imaging data were processed using FSL Software Version 5.0.11 (Jenkinson et al., 2012; Smith et al., 2004; Woolrich et al., 2009) [1]. First of all, non-brain structures were removed with the Brain Extraction Tool (BET) (Smith, 2002). Preprocessing included motion correction with MCFLIRT (Jenkinson et al., 2002), high-pass-temporal filtering to remove low frequency drifts (cutoff= 100) and spatial smoothing with an isotropic

[1] www.fmrib.ox.ac.uk/fsl

Gaussian kernel of 5mm (full width at half maximum). After the registration to MNI152 standard space, FILM prewhitening was performed. A fieldmap correction was performed to compensate for geometric distortions and signal loss of the magnetic field. If necessary, standard or extended motion parameters were added. Additional confound EVs were computed as required. The tool *fsl_motion_outliers* (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers) with the option framewise displacement root mean square (fdrms) was used to compute a confound matrix to eliminate timepoints in the fMRI dataset that have been influenced by large motion. The fdrms measure is computed from the average of displacement and rotation using the matrix root mean square formulation according to Jenkinson (2003).

3.2.4 fMRI data analysis

FMRI Expert Analysis Tool (FEAT, version 6.00) was used to perform functional MRI analysis (Woolrich et al., 2001). Data from each participant were analyzed separately at a first level of analysis. Trials for the motor task were modeled as a single factor of interest and were convolved with a canonical Gaussian hemodynamic response function and were entered as a predictor into a general linear model.

For both individual and group analyses, areas of significant fMRI responses were determined using clusters identified by a $z > 2.3$ threshold and a Threshold-Free Cluster Enhancement (TFCE)-FWE of $p < 0.05$ (Smith & Nichols, 2009; Worsley et al., 1992).

In order to allow group comparison of activation and to conduct measurements of cortical distances, all data from the right hemisphere were flipped to the left hemisphere (this method has been used for example by Diers et al., 2010; MacIver et al., 2008). For the matched controls, the activations of the left foot and left knee were accordingly flipped to the left hemisphere.

3.2.4.1 Definition of foot ROI

In neuroimaging studies, whole-brain mass-univariate voxel-wise testing of small sample sizes and the resultant reduced statistical power to detect small effects bear the risk to produce inflated or false-positive findings. An approach to mitigate these issues is the definition of a region of interest (ROI). ROIs are restricting analyses to specific brain areas that constitute a priori justified target regions (Gentili et al., 2021).

A ROI for the foot was defined based on a meta-analysis of previous task-fMRI studies generated by the software Neurosynth (Yarkoni et al., 2011). Using the search term “foot” a probabilistic map of the brain activation was derived from 83 studies. As the left hemisphere showed a higher and broader activation at visual inspection and all analyses were later performed only in the left hemisphere, only activation in the left hemisphere was considered to create the final mask. The map was thresholded at a z-score of 3. Visual inspection confirmed that the distribution of brain regions with high z-scores matched the primary motor cortex in the superior part of the precentral gyrus providing confidence that relevant brain activation locations were identified. Because we focused on the motor cortex, neural activity in other regions (frontal gyrus, frontoparietal operculum, basal ganglia, cerebellum) was eliminated manually using FSLeyes (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes) (fig. 5 & fig. 6).

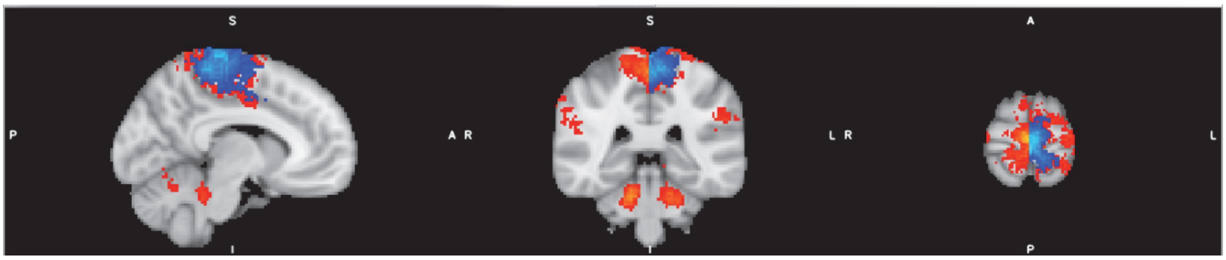

Figure 5: Definition of the foot ROI. Red: probabilistic map of the brain activation derived from the platform Neurosynth using the search term “foot” thesholded at z-score >3. Blue: Mask used in our study after manual elimination of activity in other regions than the primary motor cortex.

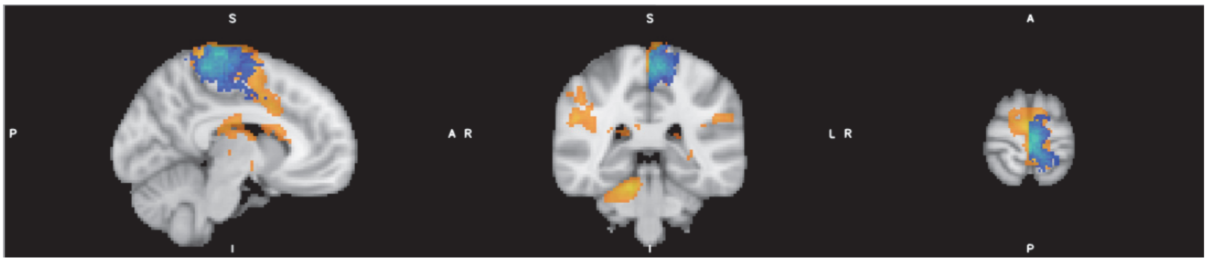

Figure 6: Overlap of the foot ROI and the mean activation map for the intact foot. Orange: Mean activation of all subjects with a rotationplasty for the intact foot. Blue: ROI used for the foot in our study. The overlap of both activation maps shows that the activation in M1 for the intact foot is covered by the ROI used.

3.2.4.2 Definition of knee ROI

We could not find a knee ROI from a metaanalysis. We therefore defined the knee ROI based on coordinates published by Shanahan et al. (2015) for the peak activation of isolated isometric contractions of the quadriceps muscle (used as a simplified model of the knee movement) performed by the participants. A spherical ROI with 10 mm radius was drawn around the peak voxel of controls x -5.0, y -31.3 and z 65.3 (Montreal Neurologic Institute (MNI) coordinates). The knee ROI was located within the previously defined foot ROI (fig. 7), thus an unbiased investigation of a possible shift of the cortical representation of the rotated foot towards the representation of the knee was possible.

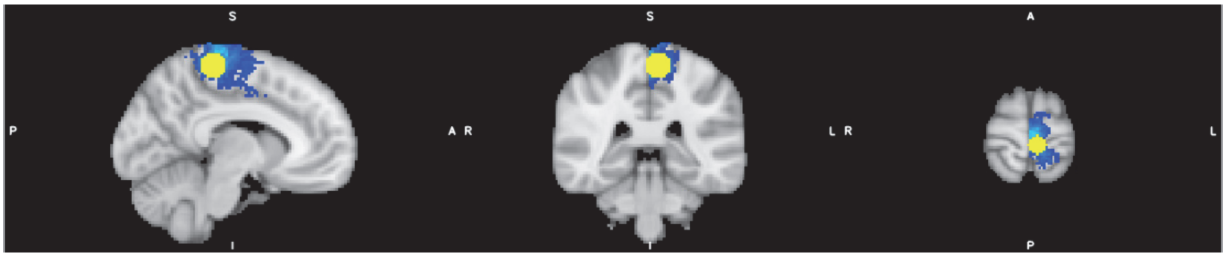

Figure 7: Overlap of the foot and the knee ROI. Blue: Foot ROI used in our study. Yellow: Knee ROI derived from Shanahan et al., 2015.

3.2.5 Intensity and extent of neural activity

Neural activity during motor movements for each body site was assessed within the masks previously defined (foot ROI for movement of the intact and rotated foot as well as for the feet of controls, knee ROI for movement of the intact knee of participants with rotationplasty and for the knee of controls) using the tool Featquery (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/UserGuide#Featquery_-_FEAT_Results_Interrogation). Intensity of neural activity was retrieved as the mean difference in fMRI signal between the baseline condition (B) and the task condition (T), referred to as percent BOLD signal change (%BSC) = (T-B)/B×100%. Additionally, the coordinates of the maximum image voxel within the ROI were obtained. The extent of neural activity was retrieved from the same analysis as the number of activated voxels within the mask.

3.2.6 Statistical analysis, somatotopy and Euclidean distances

Statistical analyses were carried out using R version 4.2.2. The data sets underwent initial assessment for normality by employing the Shapiro-Wilk test. The Shapiro-Wilk test showed that not all our variables followed a normal distribution ($p<0.05$). Equality of the variances of the samples was checked by employing the F-test. For normally distributed variables with equal variances, we conducted paired two-tailed student t-tests for within-group comparisons and unpaired t-tests for between-group comparisons. For normally distributed variables with unequal variances, we conducted Welch's t-test. Considering our sample size, we opted to use the Wilcoxon rank sum test to analyze within-group differences and the Mann-Whitney U test for between-group differences for non-normally distributed variables.

In order to investigate whether the cortical representation of the movement of the knee and the foot in subjects with rotationplasty and in controls formed distinct and significantly separated groups of somatotopic representation in the primary motor cortex, statistical comparisons between the positions of the peak voxels were performed separately for the x, y and z coordinates. The following comparisons were performed in subjects with rotationplasty: rotated foot to intact knee, intact foot to intact knee and rotated foot to intact foot. The following comparisons were performed in control subjects: right foot to right knee, left foot to left knee, right knee to left knee and right foot to left foot.

Statistical analyses of the x, y and z coordinates of the peak voxels for the knee and the foot activation in the control group did not show a significant difference between the left and right hemispheres. In consequence, symmetry of the hemispheres was assumed and further analyses were carried out without differentiation between the left and right foot as well as without differentiation between the left and right knee.

Distances between cortical representations were calculated using Euclidean distances as follows:

For subjects with a rotationplasty, cortical distances were calculated between the rotated foot and intact knee, between the intact foot and intact knee and between the rotated foot and intact foot.

For the controls, cortical distances were calculated between the left foot and left knee, between the right foot and right knee, between left and right foot and between left and right knee.

As the representation of the foot and the knee was found to be symmetrical in the left and right hemisphere in controls, the representation of the missing knee in subjects with rotationplasty was assumed to be located at the level of the representation of the intact knee.

In subjects with rotationplasty, the rotated foot fulfills the function of the knee, thus a shift of the cortical representation of the rotated foot towards the cortical representation of the knee was expected. For this reason, the distance between the rotated foot and the intact knee was predicted to be shorter than the distance between the intact foot and the intact knee. In order to test this hypothesis, statistical analyses were carried out between the cortical distances of the rotated foot/intact knee and the intact foot/intact knee.

In addition, the cortical distances between the knee and the foot in control subjects were expected to be larger than cortical distances for the same condition performed on the left and right body side (distance between the left and right foot as well as the distance between the left and right knee). Thus, statistical analyses were carried out between the cortical distances, between the foot/knee and the left foot/right foot, as well as the cortical distances between the foot/knee and the left knee/right knee.

In analogy, for participants with rotationplasty, statistical analyses were carried out between the cortical distances, between the intact foot/intact knee and the rotated foot/intact foot, as well as between the cortical distances of the rotated foot/intact knee and the rotated foot/intact foot.

Furthermore, to investigate potential differences between subjects with rotationplasty and control subjects, we compared the cortical distances between the foot/ knee in controls with the cortical distances between the rotated foot/intact knee in subjects with rotationplasty. In addition, we compared the cortical distances between the foot/knee in controls with the cortical distances between the intact foot/intact knee in subjects with rotationplasty.

4. Results

4.1 Clinical characteristics of participants with rotationplasty

Our patient cohort was homogenous in terms of reasons for rotationplasty surgery, prothesis use and phantom limb pain. All participants with rotationplasty were operated due to a tumor in the knee region. Nine out of ten participants wore the prothesis for the whole day every day. Only one participant used the prothesis almost daily but only for a few hours per day. The same participant was the only one suffering from PLP in the region of the missing knee while all other participants did not experience any PLP.

4.2 Intensity and extent of BOLD activation

The activation maps for each individual indicated neural activity in the motor cortical representation during movement of each body site. Group activation maps are shown for subjects with rotationplasty in figure 8 and for controls in figure 9.

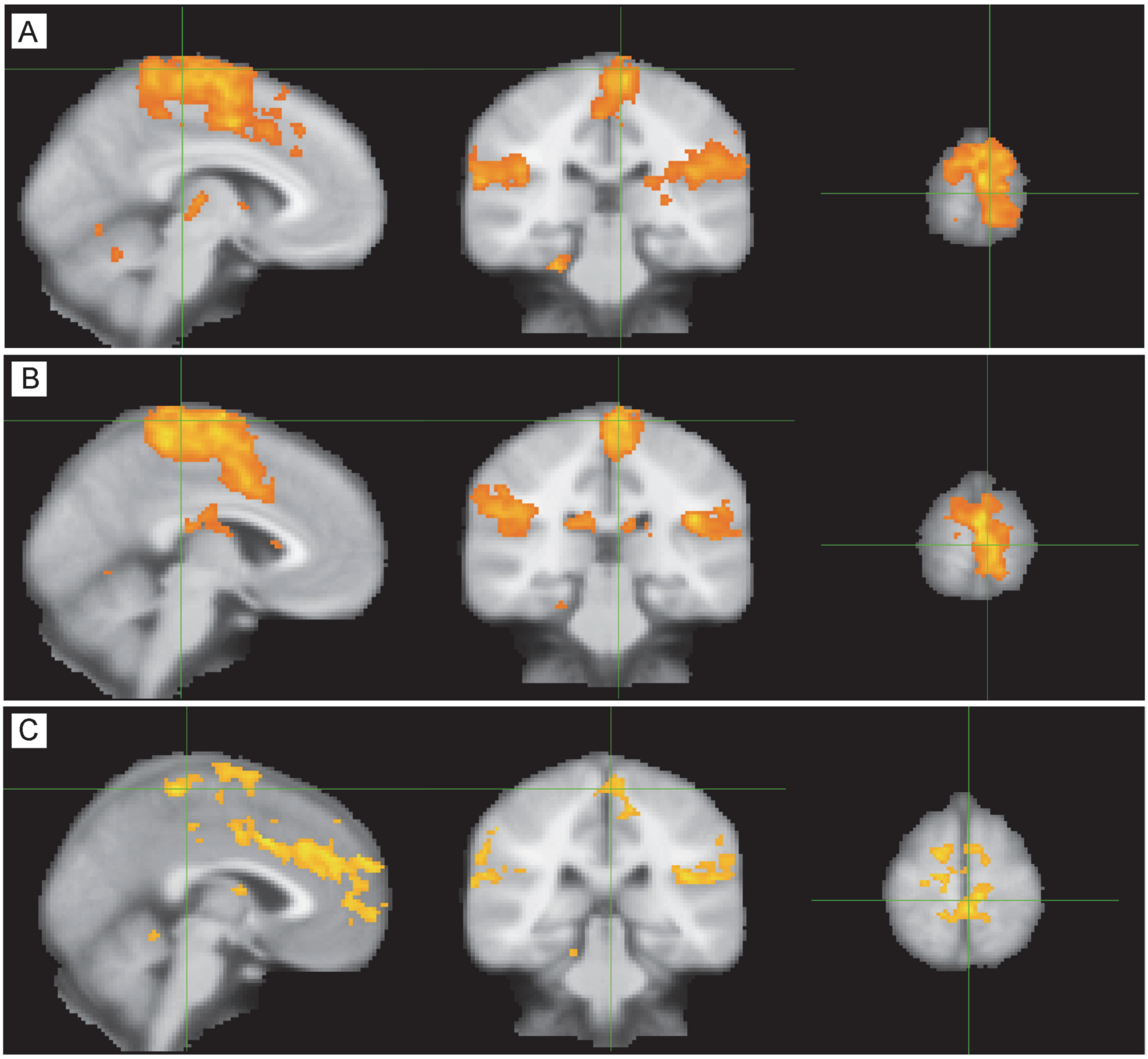

Figure 8: Group activation maps for subjects with rotationplasty moving (A) the rotated foot, (B) the intact foot and (C) the intact knee. The location cursor indicates the mean peak voxel for the respective task.

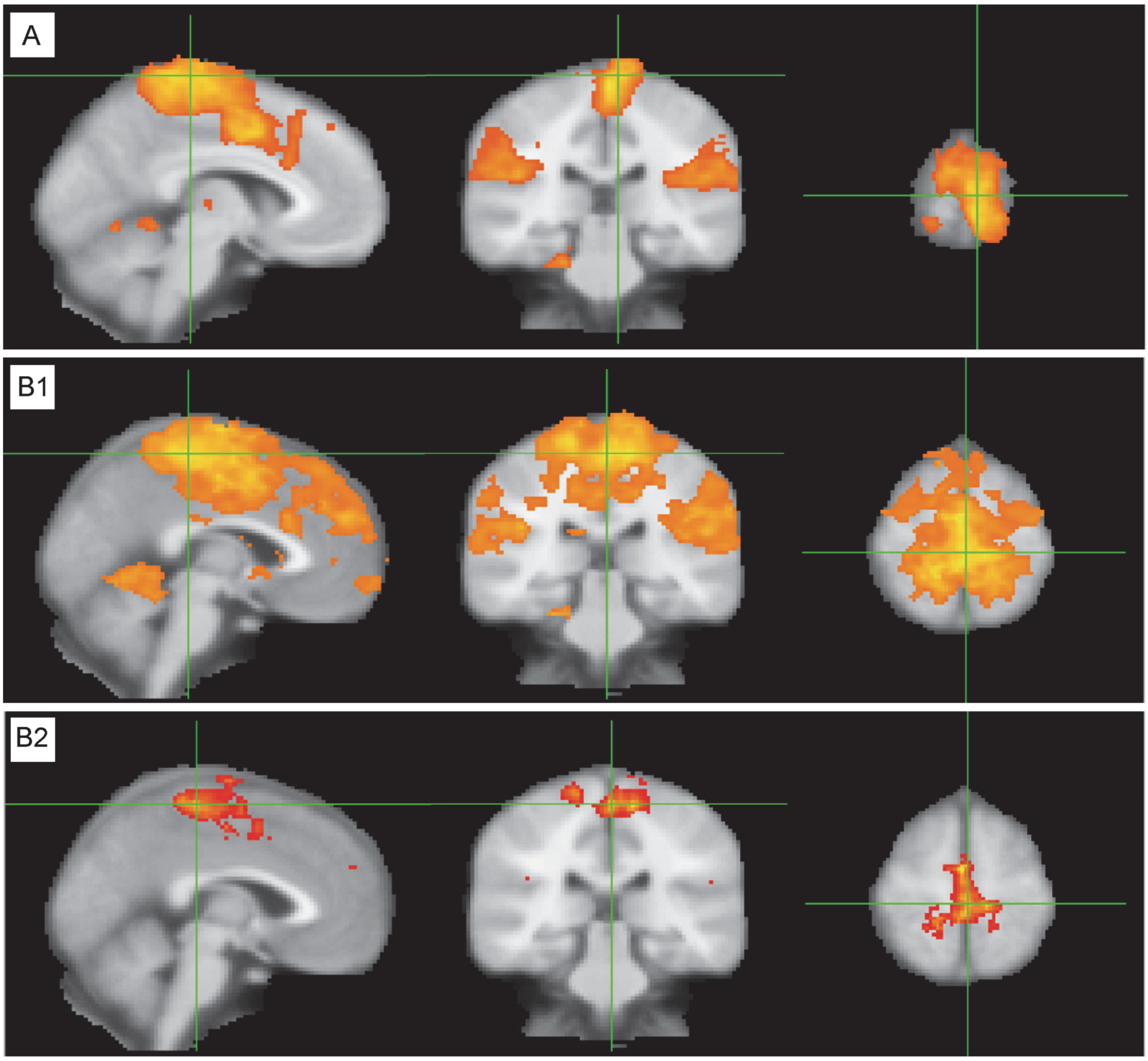

Figure 9: Group activation maps for control subjects moving (A) the foot and (B1)+(B2) the knee. (B2) Activation map for the knee movement thresholded at z-score 5 to reduce motion artifacts and visualize lateralization. The location cursor indicates the mean peak voxel for the respective task.

No significant difference in motor cortical activity (table 2) was found between the rotated foot and the intact foot ($p= 0.59$). In addition, there was no significant difference in neural activity between subjects with rotationplasty and control subjects in the foot cortex (rotated foot vs. foot control group, $p= 0.31$; intact foot in patients vs. foot in controls, $p= 0.65$). There was also no significant difference in motor cortical activity between patients and controls in the knee cortex (intact knee in patients vs. knee in controls, $p= 0.65$) as shown in figure 10.

Table 2: Activation intensities (%BSC) within the defined ROI

Group	ID	Rotated foot	Intact foot	Intact knee	
Rotationplasty	RP01	0.82	0.92	1.48	
	RP02	1.66	1.93	1.65	
	RP03	0.32	0.76	0.93	
	RP04	0.93	1.03	1.53	
	RP05	1.24	0.58	4.14	
	RP06	1.41	0.86	0.94	
	RP07	0.47	0.53	1.21	
	RP08	1.00	0.96	1.82	
	RP09	0.52	-0.16	0.51	
	RP10	0.78	0.99	2.75	
		Right foot	**Left Foot**	**Right knee**	**Left knee**
Controls	C01	0.14	0.34	0.96	1.75
	C02	0.89	0.77	1.75	1.53
	C03	0.82	0.44	1.76	1.55
	C04	0.98	0.86	2.02	1.94
	C05	1.19	0.62	1.46	1.68
	C06	0.92	0.26	2.98	1.25
	C07	0.54	0.77	0.90	0.47
	C08	0.69	1.15	1.94	1.36
	C09	0.78	0.87	0.80	1.22
	C10	0.98	1.41	1.29	1.81

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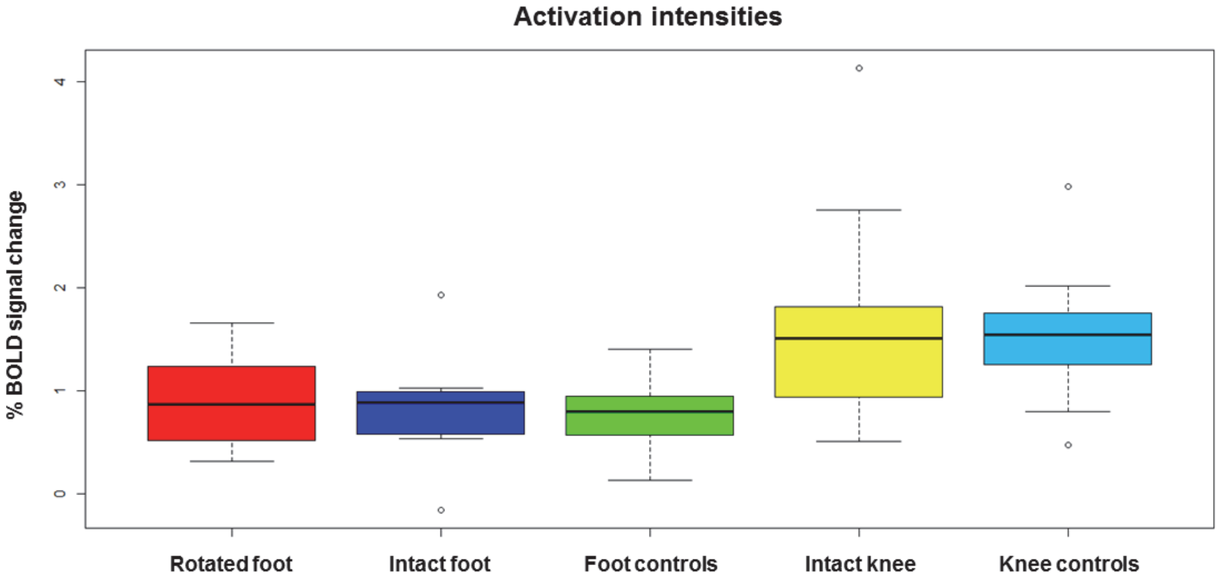

Figure 10: Medians and interquartile ranges of activation intensities (%BOLD signal change) in the contralateral motor cortex during movement of the rotated foot (red) and intact foot (dark blue) for subjects with rotationplasty, movement of the feet (left and right foot pooled, green) for controls as well as movement of the intact knee (yellow) for subjects with rotationplasty and movement of the knee (left and right knee pooled, light blue) for controls.

The extent of activation (table 3) in subjects with rotationplasty for the rotated foot and the intact foot did not show a significant difference (p= 0.40). There was, however, a trend towards significance for a difference in extent of activation between subjects with rotationplasty and control subjects for the foot movement (rotated foot vs. foot control group, p= 0.052, intact foot vs. foot control group, p= 0.059), but not for knee movement (intact knee vs. knee control group, p= 0.74) (fig. 11).

Table 3: Activation extent (number of voxels) within the ROIs

Group	ID	Rotated foot	Intact foot	Intact knee	
Rotationplasty	RP01	2702	2765	430	
	RP02	2840	2838	461	
	RP03	2911	2854	467	
	RP04	2929	2894	476	
	RP05	2953	2958	469	
	RP06	2901	2918	468	
	RP07	2866	2849	463	
	RP08	3102	3063	506	
	RP09	2821	2767	458	
	RP10	2494	2509	415	
		Right foot	**Left Foot**	**Right knee**	**Left knee**
Controls	C01	2838	3011	513	515
	C02	2158	2316	379	376
	C03	2327	2482	415	411
	C04	3153	3418	572	568
	C05	3054	3211	541	534
	C06	2456	2615	413	435
	C07	2490	2682	454	432
	C08	2721	2927	476	475
	C09	2330	2507	404	416
	C10	2065	2250	375	355

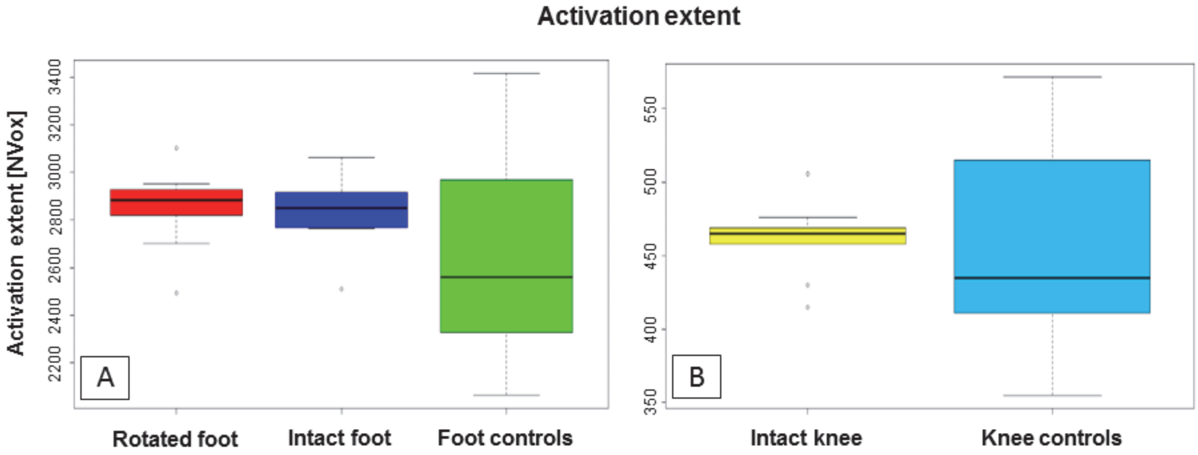

Figure 11: Medians and interquartile ranges of the extent of activation (number of non-zero voxels within the defined mask) in the contralateral motor cortex during movement of (A) the rotated foot (red) and intact foot (dark blue) for subjects with rotationplasty as well as movement of the feet (left and right foot pooled, green) for controls and for the movement of (B) the intact knee (yellow) for subjects with rotationplasty as well as movement of the knee (left and right knee pooled, light blue) for controls.

4.3 Somatotopic representation

A somatotopic organization of the foot and the knee was evident in subjects with rotationplasty and in controls. The MNI coordinates of the peak voxels for each individual are reported in table 4 and depicted in figure 12. In subjects with rotationplasty, a statistically significant separation was evident between the intact knee and intact foot (in z coordinate, p=4.5e-05) and between the intact knee and rotated foot (in x coordinate, p=0.049 and z coordinate, p=0.002). In the control group, a statistically significant separation was likewise evident between the knee and the foot in the x (p=1.7e-4) and z coordinate (p=1.9e-06).

The representation in M1 of the knee for subjects with rotationplasty as well as for control subjects was found to be located mediocaudal to the representation of the foot in M1 (fig. 12, fig. 15 & fig. 16).

In order to test the hypothesis whether the cortical representation of the rotated foot shifts towards the cortical representation of the missing knee, Euclidian distances were calculated between the peak voxels of the motor cortical representations of the rotated foot and the intact knee (which is presumably located in congruence to the missing knee) and compared to the Euclidian distances of the peak voxels of activation of the intact foot and the intact knee (fig. 13). No significant difference was found between the distance of the rotated foot to the intact knee and the distance of the intact foot to the intact knee (p= 0.14). Distances between the foot and the knee in the control group were comparable to the distances in subjects with rotationplasty (distance intact foot to intact knee vs. distance foot to knee of controls p=0.12, distance rotated foot to intact knee vs. distance foot to knee of controls p=0.81).

In analogy to the calculation of the spatial separation of the knee and the foot, the Euclidian distance between the rotated and the intact foot for subjects with rotationplasty was significantly different in comparison to the distance between the rotated foot and the intact knee (p=0.01) and significantly different in the z coordinate in comparison to the distance between the intact foot and the intact knee (p=2.8e-4). The distance between the foot and the knee for control subjects was significantly different in comparison to the distance between the right and left knee (p=3.2e-4) (fig. 14) and significantly different in the z coordinate in comparison to the distance between the right and the left foot (p=1.2e-05).

Table 4: MNI coordinates for peak activations of the rotated foot, intact foot, and intact knee in subjects with rotationplasty. MNI coordinates for peak activations of the right and left foot as well as right and left knee for control subjects. All peak activations are presented in the left hemisphere.

Group	ID	X(M1)	Y(M1)	Z(M1)	X(M1)	Y(M1)	Z(M1)	X(M1)	Y(M1)	Z(M1)	X(M1)	Y(M1)	Z(M1)
		Rotated foot			Intact foot			Intact knee					
Rotationplasty	RP01	0.2	-34.3	70.7	-1.3	-27.8	70.2	-1.3	-26.8	67	N/A		
	RP02	-5	-31	78.9	-11.5	-28.9	78	-2.2	-40.4	67.8			
	RP03	0	-23.1	72.8	-7.7	-19	77.9	-2.7	-31.7	71.5			
	RP04	-9.8	-43.5	79.1	-3.8	-33.7	77.4	-2.5	-36.4	71.8			
	RP05	-1.5	-25.6	67.3	1.1	-24.7	68.5	0.1	-26.1	60.1			
	RP06	-3.1	-22.9	79.3	-0.4	-32.1	74.7	1	-36.8	61.2			
	RP07	-6.7	-13.5	78	-3.9	-24.3	79.8	-9.1	-23.3	68.4			
	RP08	-8.6	-34.6	79.4	-10.4	-34.8	79.4	0.1	-26	62.3			
	RP09	-6.6	-21.4	79.7	-1.2	-16.8	75.3	-1.6	-37.4	62.6			
	RP10	-15.6	-28.2	73.2	-4.5	-45.8	68.6	-2.7	-29.6	60.7			
		Right foot			Left Foot			Right knee			Left knee		
Controls	C01	-4.2	-21.9	66.6	-5.5	-18.9	79.3	-0.6	-24.4	64.7	1.1	-29.5	65
	C02	-3.1	-31.8	61	-6.4	-32.9	69.4	-2.1	-32.7	55.2	0.3	-30	57.6
	C03	-6.4	-35.2	79.8	-0.6	-30	76.7	-3	-31.2	56.5	2.7	-29.1	59.8
	C04	-8	-31.8	78	-7.8	-11.5	79.4	-1.5	-31	62.4	1.3	-30.4	62.4
	C05	-12.7	-31.5	80.8	-3.3	-8.3	74.3	0.5	-25.3	67.5	-0.9	-24.4	64.4
	C06	-15.9	-44.5	79.7	-6.5	-31.7	79	-0.2	-26.1	59.2	-1.4	-36.7	56.9
	C07	-12.7	-45.9	79.1	-4.2	-29.5	77.7	-0.4	-28	62	1.6	-26.9	65.9
	C08	-3	-4.6	74.8	0.4	-4.6	67.1	-7.3	-26.6	71.9	-1.9	-27.8	58.5
	C09	-1.4	-31.4	75.6	-7.8	-28	79.6	0.5	-31.6	68.1	-1.6	-27.4	64.8
	C10	-8.5	-38.6	78.8	-11.8	-46	74.2	-1.2	-25.1	59.3	-9.7	-37.1	70

Table 5: Mean MNI coordinates for peak activations. All peak activations were calculated in the left hemisphere. SD = standard deviation.

Group	Condition	X(M1) ± SD	Y(M1) ± SD	Z(M1) ± SD
Rotationplasty	Rotated foot	-5.7±4.7	-27.8±8.0	75.8±4.2
	Intact foot	-4.4±4.0	-28.8±8.0	75.0±4.1
	Intact knee	-2.1±2.6	-31.5±5.6	65.3±4.3
Controls	Foot	-6.5±4.2	-27.9±12.4	75.5±5.3
	Knee	-1.2±2.8	-29.1±3.6	62.6±4.6

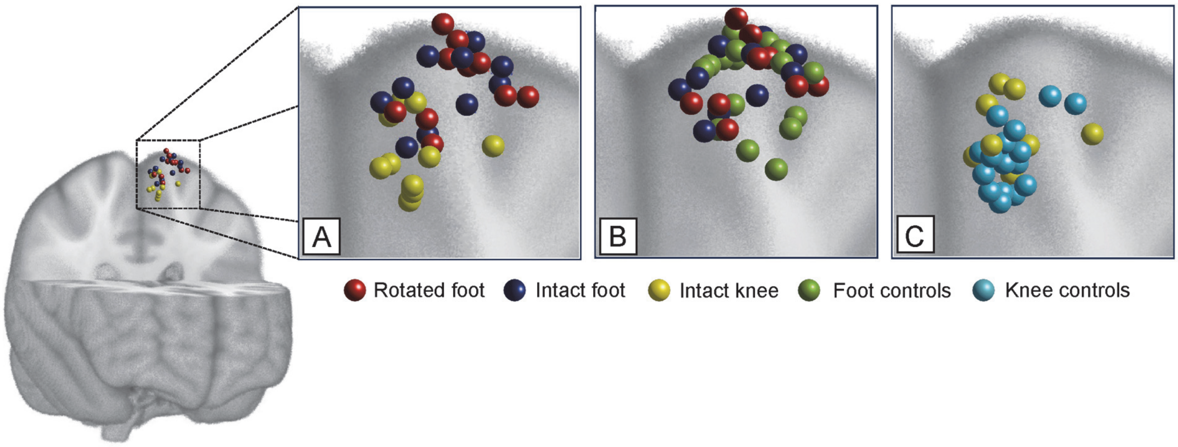

Figure 12: ROIs based on the peak voxels for (A) rotated foot (red), intact foot (dark blue) and intact knee (yellow) in patients with rotationplasty. (B) Rotated foot (red), intact foot (dark blue) in patients with rotationplasty and foot in controls (green). (C) Intact knee (yellow) in patients with rotationplasty and knee in controls (light blue).

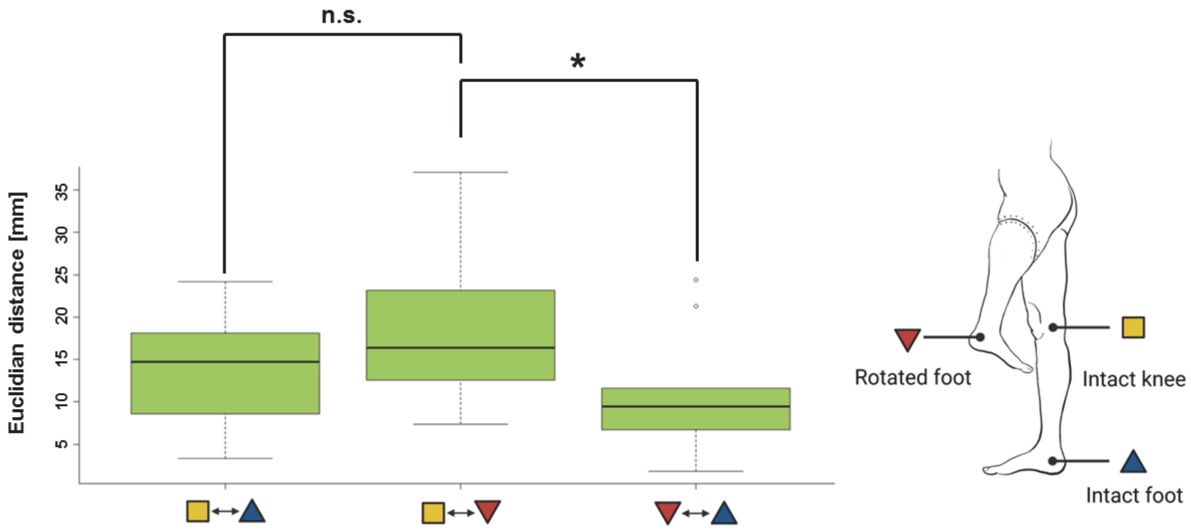

Figure 13: Medians and interquartile ranges of Euclidean distances in mm calculated for the distance between the intact knee (yellow square) and intact foot (blue triangle), the intact knee and the rotated foot (inverted red triangle), the rotated foot and the intact foot for subjects with rotationplasty. Significant differences were found between the distance of the rotated to the intact foot in comparison to the distance of the rotated foot to the intact knee (p=0.01) as well as in comparison to the distance of the intact foot to the intact knee in the z coordinate (p< 0.0003). n.s. = non-significant; * = p-value < 0.05.

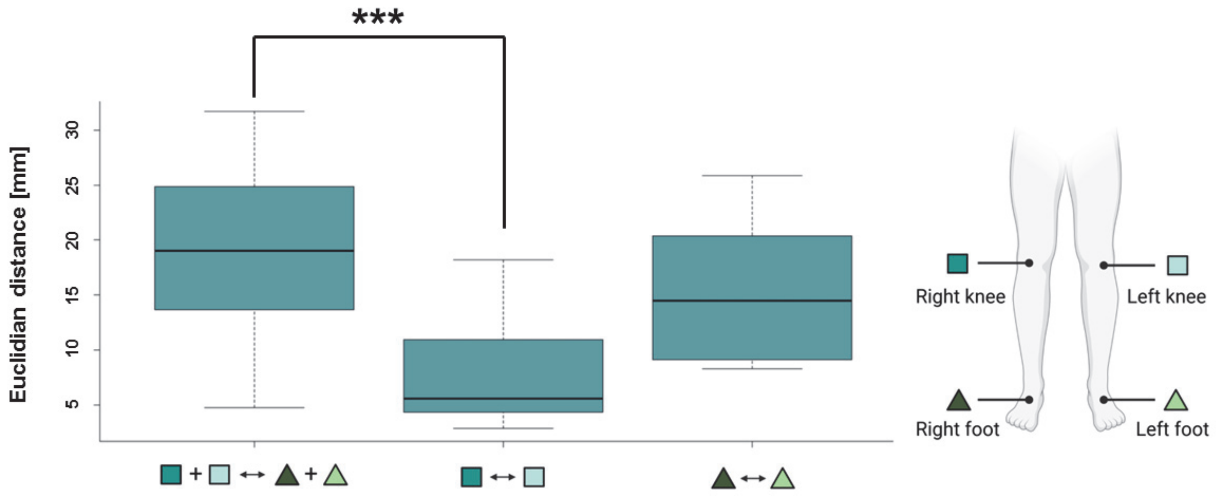

Figure 14: Medians and interquartile ranges of Euclidean distances in mm calculated for the distance between the pooled knees (right knee: dark blue square, left knee: light blue square) and the pooled feet (right foot: dark green triangle, left foot: light green triangle), the right and the left knee, the right and the left foot for control subjects. Significant differences were found between the distance of the foot to the knee in comparison to the distance of the right and left knee (p=3.2e-4) as well as in comparison to the distance of the right to the left foot in the z coordinate (p=1.2e-05). *** = p-value < 0.001. Created with BioRender.com.

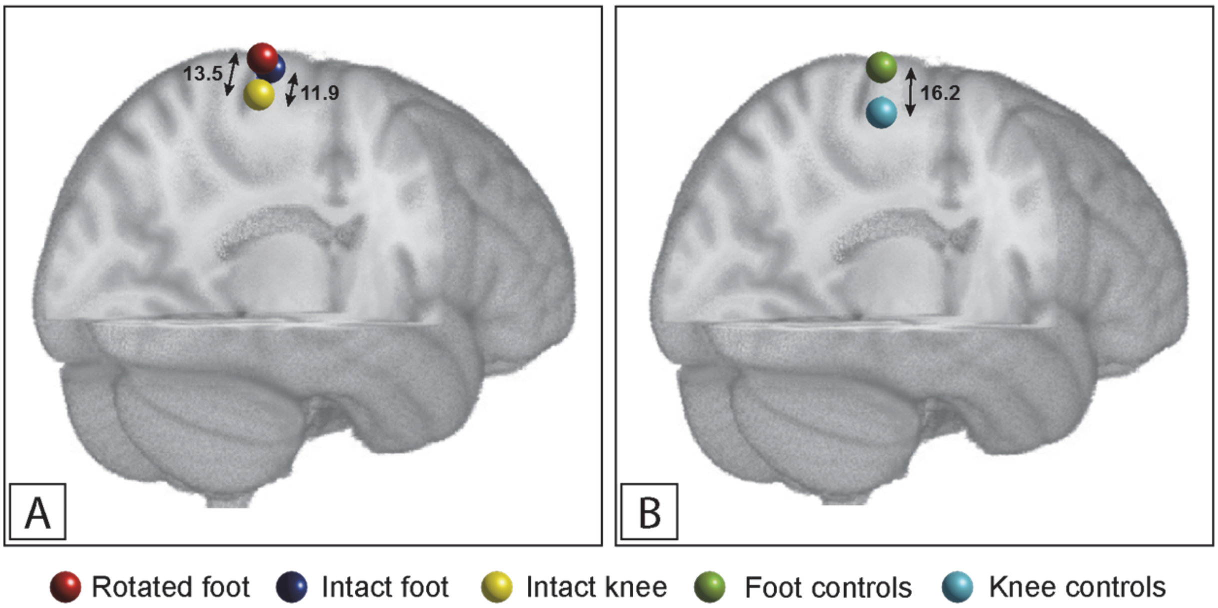

Figure 15: ROIs (diameter 4mm) based on the mean of the individual ROIs for (A) rotated foot (red), intact foot (dark blue) and intact knee (yellow) in subjects with rotationplasty and (B) foot (green) and knee (light blue) in control subjects. Double-headed arrows indicate cortical distances in mm. ROIs are mapped on a MNI152 template provided by FSL.

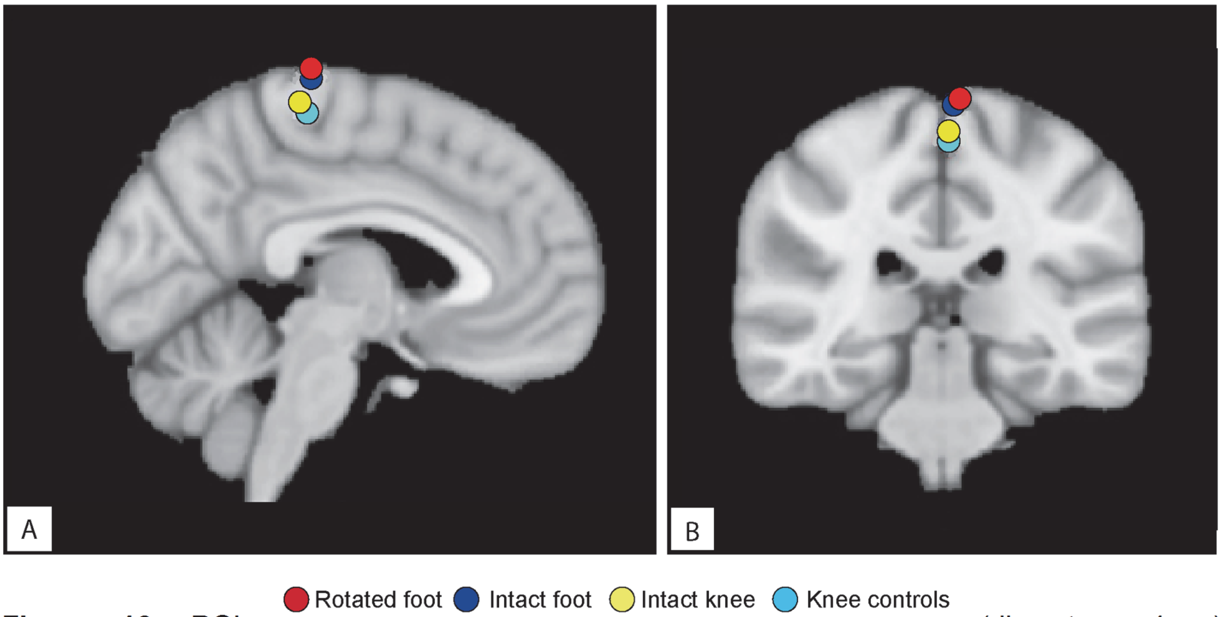

Figure 16: ROIs (diameter 4mm) based on the mean of the individual ROIs for rotated foot (red), intact foot (dark blue), intact knee (yellow) and knee of controls (light blue) in (A) sagittal and (B) coronal view. The ROI for the foot of controls is not depicted as it entirely overlaps with the ROI of the rotated foot.

On visual inspection, the peak voxels of the rotated foot appeared more dispersed than the peak voxels of the intact foot. To quantify whether the peak voxels of the rotated foot were more dispersed than the peak voxels of the intact foot or the foot of control subjects, Euclidian distances of each peak voxel to the mean peak voxel of the respective task (table 5) were calculated. No significant difference was evident between the groups, thus the dispersion of the peak voxels of the rotated foot is comparable to the dispersion of the intact foot as well as to the foot of control subjects. The dispersion of the peak voxels is visualized by scatterplots grouped by concentration ellipsoids with a 95% confidence interval shown in figure 17. The dispersion of the peak voxels for each task was additionally quantified by calculation of the volume of the concentration ellipses (table 6).

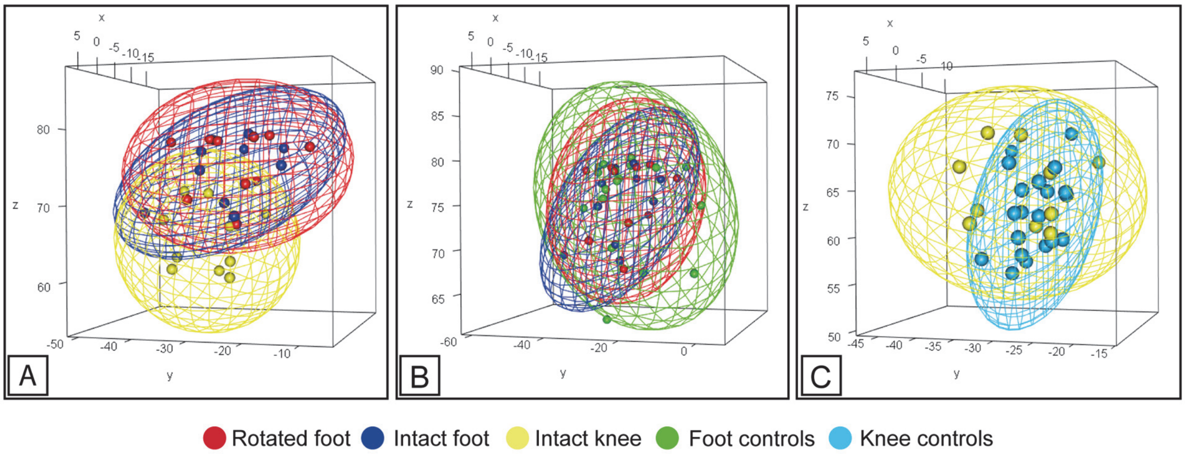

Figure 17: Scatterplots based on the peak voxels for (A) rotated foot (red), intact foot (dark blue) and intact knee (yellow) for subjects with rotationplasty. (B) Rotated foot (red), intact foot (blue) and feet of controls (green). (C) Intact knee (yellow) and knee of controls (light blue). Peak voxels are grouped by ellipses with confidence interval of 95%.

Table 6: Volumes of concentration ellipses depicted in figure 17 normalized by the volume of the concentration ellipse of the rotated foot

Condition	Normalized volume
Rotated foot	1
Intact foot	0.63
Foot controls	1.17
Intact knee	0.34
Knee controls	0.25

Table 7: Overlap between the activation extent of the rotated foot and the intact knee within the knee ROI as well as overlap between the activation extent of the intact foot and the intact knee within the knee ROI. For this calculation, the number of voxels activated by movement of the rotated foot or intact foot, respectively, within the knee ROI yielded from the conjunction analysis was divided by the number of voxels activated by movement of the intact knee within the knee ROI.

Subject	Overlap activation extent of rotated foot and intact knee within knee ROI	Overlap activation extent of intact foot and intact knee within knee ROI
RP01	1.05	1.06
RP02	1.00	1.00
RP03	0.80	0.93
RP04	0.91	0.92
RP05	0.98	0.97
RP06	0.93	0.93
RP07	0.81	0.90
RP08	0.83	0.91
RP09	0.95	0.65
RP10	1.00	1.07

The peak location of activation remains in the foot area as shown by within-subject analysis. However, a change in the configuration of the area of activation elicited by movement of the rotated foot could lead to an invasion of the cortical representation of the rotated foot into the area of cortical representation of the missing knee. Conjunction analysis confirmed that most voxels within the knee ROI were activated by movement of the rotated foot as the knee ROI is located within the foot ROI. Likewise, most voxels within the knee ROI were activated by movement of the intact foot (table 7).

In order to examine whether movement of the rotated foot led to a higher cortical activation within the knee ROI in comparison to movement of the intact foot, cortical activation (%BSC) was calculated from the knee ROI during movement of the rotated and the intact foot. Statistical analysis revealed no significant difference in %BSC within the knee ROI between the movement of the rotated foot and the movement of the intact foot ($p=0.53$) (fig. 18). Thus, no evidence for an invasion of the area of the cortical representation of the rotated foot into the area of the missing knee was found.

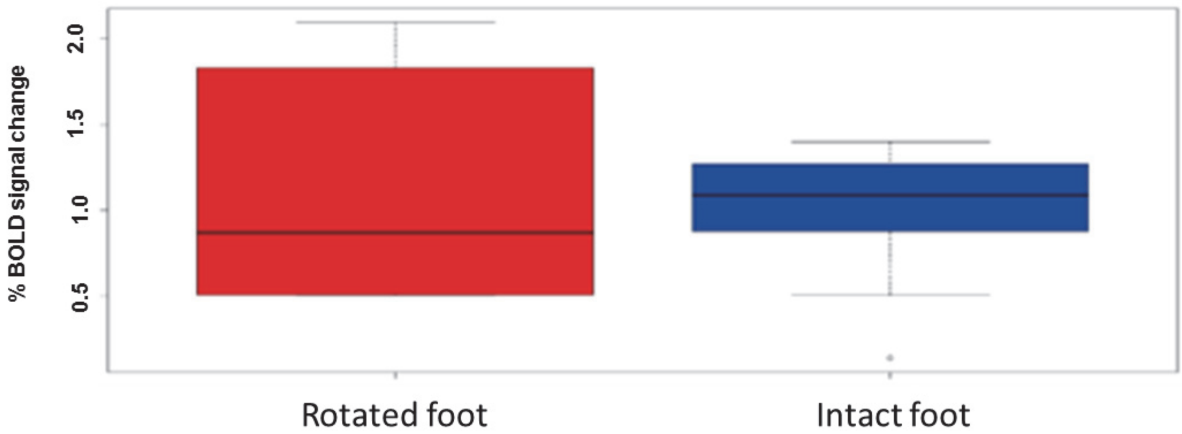

Figure 18: Medians and interquartile ranges of activation intensities (%BOLD signal change) extracted from the knee ROI in the contralateral motor cortex during movement of the rotated foot (red) and intact foot (blue) for subjects with rotationplasty. No significant difference was found.

5. Discussion

5.1 Somatotopic sequence of the leg representation in M1

All subjects showed increased neural activity in the mesial surface of the precentral gyrus upon movement of the lower extremity, consistent with the cortical motor homunculus theory. However, the representation of the foot was found superio-lateral to the representation of the knee in subjects with rotationplasty as well as in the control group, which is not in line with the cortical motor homunculus theory that predicts the knee to be found superio-lateral to the representation of the foot.

Discrepancies between the classical motor homunculus and somatotopic maps of the primary motor cortex for the lower extremities yielded by fMRI studies have previously been described in the literature. Kapreli et al. (2007) found the foot to be superior and medial to the knee representation, thus only in part reproducing the somatotopic map of the lower extremity predicted by Penfield and Rasmussen (1950), whereas Shanahan et al. (2015) found the foot to be represented anterior and superior to the knee, while no shift in the medio-lateral direction was noted. These deviations of fMRI study results from Penfield’s homunculus might originate from methodological differences. Indeed, Penfield’s homunculus was obtained invasively via direct and focal cortical stimulation (Penfield & Boldrey, 1937) while the cortical maps yielded via fMRI are obtained indirectly by measuring the BOLD signal change, thus tracing an increased oxygen demand of a network of cortical areas involved in the execution of a movement.

Moreover, Penfield’s homunculus does not reflect the vast overlap of neighboring motor cortical representations of the lower extremities shown in the present study as well as in the literature (Kapreli et al., 2007). Indeed, direct cortical stimulation of the areas described by Penfield and Rasmussen elicited movements in the knee or the leg, while cortical activations obtained using fMRI during movements of the knee or leg might be confounded by additional processes, such as decision making and sensory feedback.

Beyond that, the spatial resolution of the leg representation in Penfield’s homunculus is restricted by the difficult accessibility of the mesial surface of the precentral gyrus for direct stimulation, thus further contributing to discrepancies between the cortical organization of the leg predicted by the classical homunculus and the one found by fMRI.

Incongruities between the present study and published data regarding the representation of the knee relatively to the ankle might also be explained by methodological differences in the experimental procedure and analysis methods. For instance, in the study conducted by Kapreli et al. (2007), fMRI was carried out while participants performed isolated joint movement of the knee or the ankle, similarly to the experimental setup in our study. However, task-related neural activity was analyzed using the center of mass, whereas we used peak voxels. Given that the area of activation during knee and foot movement is shaped asymmetrically rather than spherically (fig. 8 and fig. 9) and that the shape and extent of activation are influenced by motion artifacts, peak voxels are considered to present a more reliable representation of the activation in the present study.
Additional differences between studies could arise from the motor task being performed. In Shanahan et al. (2015), participants were asked to perform submaximal isometric contractions of the quadriceps to represent knee movements and submaximal isometric contractions of the tibialis anterior to represent ankle movements. In the present study, participants were performing isotonic movements of the respective joint, which might likely lead to a slightly different representation of the foot in M1 in comparison to Shanahan et al. (2015).

5.2 Maintained representation of the rotated foot

Motor training is known to be a driver of cortical plasticity, though fMRI research has demonstrated that motor learning can result in both activity increases and decreases in primary and secondary motor areas (Dayan & Cohen, 2011; Hardwick et al., 2013). On the one hand, motor skill acquisition may increase average activation via increased neural recruitment for trained behaviors (Karni et al., 1995; Floyer-Lea & Matthews, 2005; Penhune & Doyon, 2005). On the other hand, motor skill acquisition may decrease average neural activity by developing neural representations that produce the same behavior with higher neural efficiency (Toni et al., 1998; Ungerleider et al., 2002; Xiong et al., 2009; Penhune & Doyon, 2005).
Previous studies have shown a loss of muscular strength of the rotationplasty in comparison to the contralateral intact ankle (Steenhoff et al., 1993, Murray et al., 1985) as well as a reduced extensor mechanism of the rotationplasty in most of the patients (Hillmann et al., 2000).

A decreased muscular strength and an impaired motion pattern of the rotated foot in subjects with rotationplasty is likely to be associated with an asymmetrical limb usage. Thus, we expected decreased neural activity in M1 cortex of the cortical representation of the rotated foot in comparison to the one of the intact foot. However, the present study showed no significant differences in terms of neural activation intensity and extent between the cortical representation of the rotated foot and the intact foot.

Preservation of neural activation intensity and extent of the rotated foot may result from the high functionality of the rotated foot following rotationplasty leading to a relatively normal walking pattern despite an expected asymmetry in limb usage. Moreover, patients with rotationplasty receive constant peripheral input from the operated limb which is vital for the formation and stability of the cortical sensorimotor map (Serino et al., 2017).

Furthermore, the cortical representation of the rotated foot in subjects with rotationplasty remains in the foot area, and not in the knee area, as shown by within-subject analysis as well as in comparison to the control group. A maintained cortical representation after amputation has been shown in some studies (Makin et al., 2013). However, studies reporting maladaptive cortical reorganization have been shown in amputees with PLP (Grüsser et al., 2001; Flor et al., 1998; Wu & Kaas, 1999; Schwenkreis, 2001; Mercier & Léonard, 2011; Karl et al., 2001; Karl et al., 2004; Lotze et al., 2001). Most individuals with rotationplasty from our cohort did not report PLP with only one individual suffering from low PLP, which may explain why there was no cortical reorganization. Moreover, extensive wearing time and use of a myoelectric prothesis has been shown to be associated with less cortical reorganization (Lotze et al., 1999). In addition, forearm amputees using a prosthesis that provides somatosensory feedback on the grip strength suffer less from phantom limb pain (Dietrich et al., 2012), thus presumably show reduced cortical reorganization. Furthermore, higher motor control of the phantom limb has been correlated with less PLP (Kikkert et al., 2017) and is, in consequence, likely as well correlated with less cortical reorganization.

While subjects with rotationplasty and leg amputees share a significantly altered configuration of the body, as a part of the lower extremity is missing, both groups are distinguished by a range of specific anatomical and functional features.

-One major anatomical difference consists in the intact peripheral nervous system in subjects with rotationplasty, whereas peripheral nerves have to be resected in the course of amputation. Thus, subjects with rotationplasty receive tactile and proprioceptive feedback from the operated leg via preserved afferents while amputees often experience impaired or even painful sensations of the stump (Jensen et al, 1983; Kosasih & Silver-Thorn, 1998; Hsu & Cohen, 2013).

Compared to above-knee amputees, patients with rotationplasty show several functional advantages including a better knee-control and an increased weightbearing capacity of the foot (Fuchs et al., 2003) leading to a fairly normal walking pattern (Hillmann et al., 2000) and often a better ability to engage in athletic activities (Bernthal et al., 2014). Data from our cohort indicate that most individuals with rotationplasty use the prothesis for all daily activities.

In summary, factors associated with preservation of the cortical representation of the missing limb in amputees, namely low phantom limb pain, frequent prothesis use, sensory feedback from the prothesis and a high level of motor control are highly prevalent in subjects with rotationplasty. In addition, the rotated foot functioning as a knee remains to be controlled by the same nerves and muscles that controlled the foot at the position of the ankle. These factors presumably prevent an invasion of the cortical representation of the rotated foot into the area representing the resected knee.

Tesio et al. (2014) showed a spatial overrepresentation of the unrotated soleus and vastus medialis via TMS. Transferring this finding to the present study, a spatial overrepresentation of the intact foot and intact knee would be expected. However, we did not find any significant differences between the spatial representation of the intact foot and the rotated foot using either activation extent or conjunction analysis.

Moreover, there was no significant difference between the spatial representation of the movements of the intact joints in subjects with rotationplasty and controls. Differences between the present study and Tesio et al. (2014) could be related to the small sample size. In Tesio et al., three individuals with rotationplasty were enrolled, whereas we enrolled 10 participants. Furthermore, comparisons between the unaffected and operated side as well as comparisons between subjects with rotationplasty and healthy controls did not reach statistical significance in Tesio et al.’s study.

5.3 Limitations of this study and future work

The power of this study is limited by the small size of the cohort consisting of ten subjects with rotationplasty and ten controls, which is, nevertheless, higher than other studies investigating individuals with rotationplasty (e.g., n= 3 in Tesio et al., 2004).

Studies using fMRI for the investigation of cortical organization in amputees (Makin et al., 2013; Andoh et al., 2020) or in unimpaired subjects (Akselrod et al., 2017; Dietrich et al., 2017; Kapreli et al., 2007) have around 15-20 participants, other studies including Raffin et al. (2016) or Shanahan et al. (2015) used comparable cohort sizes with 11 participants or even less like Willoughby et al. (2020) with seven participants. While cohort size plays a role in the analysis of changes on a group level, it does not limit the investigation of possible differences on an individual level such as the analysis of inter-hemispheric changes.

Performing lower limb movements might inadvertently cause head movements. As small head movements can cause large variations of the signal, movement has to be taken into account as a main confounder. While care has been taken to train the participants to execute leg movements as smoothly and stable as possible, head motion could not be completely prevented. Movement artifacts are particularly problematic in MRI scans with low signal-to-noise ratios as in fMRI, especially if they are correlated with the experimental paradigm as in our study. Thus, motion artifacts have been considered as a main confounder by application of motion correction algorithms during preprocessing.

The advancement of standard field strength 3T MRI to ultra-high field of 7T and above allows for an increased sensitivity and greater spatial resolution. Considering the proximity of the representation of neighboring regions of the lower extremity and a vast overlap of representation, ultra-high field fMRI might contribute to a deeper understanding of cortical reorganization after rotationplasty surgery.

In order to assess cortical reorganization, we measured Euclidean distances between peak activations resulting from volume based analyses. Such analysis methods consist in measuring linear distances between two brain regions and have been used in comparable studies (Kapreli et al., 2007; Andoh et al., 2020).

However, alternative methods using geodesic distances which take into account individual folding patterns are rousing growing interest since some studies suggest that they may provide more accurate measurements (Jo et al., 2007; Maeda et al., 2014). Nevertheless, both Euclidean and geodesic distances have been demonstrated to be significantly correlated in M1 and S1 as well as in the sensorimotor cortex (Jo et al., 2007; Makin et al., 2015). Furthermore, distance changes between the local maxima of M1 and S1 were not significant when comparing volume- and surface-based analyses (Jo et al., 2007).

While the present study provides insights into the representation of the rotated foot in the primary motor cortex, questions remain unsolved regarding what happens to the cortical area representing the missing knee as well as the representation of the rotated foot in the primary sensory cortex. Mapping not only the sensory representation of the rotated foot but also by sensory stimulation of adjacent areas in analogy with experiments performed on amputees (Ramachandran et al., 1992; Flor et al., 1995; Grüsser et al., 2001) could provide insights into cortical reorganization of the rotated foot as well as information on the representation of the missing knee.

Questions raised by Tesio et al. (2014), whether the rotated soleus retains its original cortical representation or whether its cortical representation is taken over by cortical areas originally focused on the vastus medialis, could only in part be solved by our study, as the study design focused on isolated joint movement rather than isolated muscle contraction. While the results of the present study are pointing to a maintained cortical representation of the soleus muscle functioning as a vastus medialis, experiments requiring isolated isometric muscle contraction are needed to gain further insight.

Open questions remain regarding the embodiment of the rotated foot. Whether individuals with a rotationplasty continue to perceive the rotated foot as a foot or whether the subjective perception shifts towards a knee warrants further investigation.

6. Summary

The investigation of cortical reorganization in amputees relies on surrogate parameters such as motor imagery, phantom limb movement or sensory stimulation of adjacent areas. In contrast, the unique group of subjects with a rotationplasty allows for direct mapping of the cortical representation of the rotated foot which functions as a knee while the central and peripheral nervous systems remain intact. We used functional magnetic resonance imaging to study the leg's neural representation in the primary motor cortex. Ten subjects with rotationplasty and ten matched controls performed isolated flexion/extension movements of the knee, the ankle and the rotated foot in a block-design experiment.

A somatotopic organization of the knee and the foot was evident. However, the arrangement diverged from Penfield and Rasmussen's homunculus as the representation of the foot was found superio-lateral to the representation of the knee in subjects with rotationplasty and in controls. We show that the cortical representation of the rotated foot is preserved as a foot instead of adopting a cortical representation as a functional knee in terms of activation intensity, extent and configuration. Moreover, the cortical representation of the rotated foot remains at the position of the foot in the primary motor cortex rather than shifting towards the knee representation. Analogously to findings in amputees, low phantom limb pain, frequent prothesis use, sensory feedback and a high level of motor control might prompt preserved cortical representation in subjects with rotationplasty.

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PEER REVIEWED PUBLICATIONS

1. **Doubrovinskaia S**, Sahm F, Thier MC, Bendszus M, Wick W, Seliger C, Kaulen LD: Primary CNS lymphoma after CLIPPERS: a case series. J Neurol Neurosurg Psychiatry, 92(12):1348-1349, 2021

2. Kaulen LD, **Doubrovinskaia S**, Mooshage C, Jordan B, Purrucker J, Haubner C, Seliger C, Lorenz HM, Nagel S, Wildemann B, Bendszus M, Wick W, Schönenberger S: Neurological autoimmune diseases following vaccinations against SARS-CoV-2: a case series. Eur J Neurol, 29(2):555-563, 2022

3. Doubrovinskaia S, Mooshage C, Seliger C, Lorenz HM, Nagel, Lehnert P, Purrucker J, Wildemann B, Bendszus M, Wick W, Schönenberger S, Kaulen LD: Neurological autoimmune diseases following vaccinations against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A follow-up study. Eur J Neurol, 30(2): 463–473, 2023

PRESENTATION

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AWARDS AND HONORS

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