# New MRI-based methods for the identification of cortical and subcortical eloquent brain areas
# Implementations in pediatric patients

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PhD Thesis

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# Implementations in pediatric patients

PhD Thesis Summary

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## Original publications directly related to the thesis:

**I. Máté A**, Lidzba K, Hauser TK, Staudt M, Wilke M: A "one size fits all" approach to language fMRI: increasing specificity and applicability by adding a self-paced component. Exp Brain Res. 2016 Mar;234(3):673-84.

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**II. Máté A**, Kis D, Czigner A, Fischer T, Halász L, Barzó P: Connectivity-based segmentation of the brainstem by probabilistic tractography. Brain Res. 2018 Jul 1;1690:74-88.

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## Abbreviations

**3D IR-FSPGR** - 3D inversion recovery-prepared fast spoiled gradient-echo; **ALIC** - anterior limb of the internal capsule; **ARAS** - ascending reticular activating system; **BL** - block; **CDR** - connectivity-defined region; **CM** - connectivity map; **$CoG_{conn}$** - center of gravity of the connectivity values; **CST/CBT** - corticospinal and corticobulbar tracts; **DTI** - diffusion tensor imaging; **EPI** - echo planar imaging; **ER** - event-related; **FA** - fractional anisotropy; **FDR** - false discovery rate; **FDT** - FMRIB's diffusion toolbox; **FLAIR** - fluid-attenuated inversion recovery; **fMRI** - functional magnetic resonance imaging; **FMRIB** - Oxford Centre for Functional MRI of the Brain; **FSL** - FMRIB's software library; **FWE** - family-wise error; **LI** - lateralization index; **MNI152** - Montreal Neurological Institute 152; **PDM** - probability distribution map; **PLIC** - posterior limb of the internal capsule; **PPVT** - Peabody picture vocabulary test; **SOA** - stimulus onset asynchrony; **SYN** - synonyms task; **SWI** - susceptibility-weighted imaging; **TBI** - traumatic brain injury; **VIT** - vowel identification task

The aim of my research was the elaboration of non-invasive MRI-based imaging methods that potentially advance the diagnostic and therapeutic approaches of many diseases of the central nervous system. My PhD work covered two main fields: the methodological improvement of a pediatric fMRI language task battery and connectivity-based segmentation of the brainstem by probabilistic tractography. Considering the different methodology and character of these works, they are discussed in separate chapters below.

## Self-paced paradigm and event-related analysis for increased specificity and applicability of pediatric language functional MRI

### Introduction

Noninvasive language mapping by functional magnetic resonance imaging (fMRI) is increasingly used to identify the language-dominant hemisphere in the context of presurgical evaluation. However, in pediatric patients using fMRI brings about numerous practical and methodological issues. Special attention should be paid to task design to adapt the level of difficulty to the varying abilities of the participating age and patient groups.

To assess “hemispheric dominance” in language processing the Experimental Pediatric Neuroimaging Group at the University of Tuebingen has previously elaborated an fMRI task battery, which is well applicable in children with average language abilities: the child-appropriate versions of the synonyms task (SYN) and the vowel identification task (VIT).

In the original child-appropriate version of the task battery both tasks are used with a 5-second fixed stimulus onset asynchrony ([SOA] i.e., 5 seconds between the onset of two stimuli), and data analyses are performed in a block (BL) design, that is stimuli of the active and control conditions are presented in alternating blocks. These tasks were shown to produce robust activations in the inferior frontal and posterior temporal language areas in the dominant hemisphere in children with average language skills. However, children with above-average language functions found the 5-second fixed SOA too slow, likely leading to “mind wandering”. In contrast to this, for patients with lower language abilities the paradigm was too fast, leading to frustration and loss of compliance. Therefore, this task design was not suitable for participants with language abilities outside the average range.

I had the opportunity to take part in the study conducted at the University of Tuebingen to adapt the SYN and the VIT to participants with lower- as well as higher-than-average language functions, thereby broadening the applicability of this task battery. To keep the modified tasks comparable to the original versions, the optimal solution to adapt them to the varying language abilities of the participants is the implementation within a self-paced paradigm; here, the participant individually determines the appearance of the next stimulus, as it only appears after his/her response.

In addition to the self-paced modification, the specificity of the tasks could be increased further by using an event-related (ER) analysis of the data, as it may provide a better model for the transient changes in the neural activity. A drawback of such designs is the reduced design efficiency as compared with a BL design paradigm.

In the language domain in particular, a lateralization index (LI) is commonly calculated to determine hemispheric specialization. As a paradigm that yields more specificity in the detection of task-related activations must be expected to reveal more focused (and thus likely more lateralized) brain activity, assessing the lateralization of activation obtained in the BL and ER analyses may be helpful when comparing the two analytical approaches in this regard.

**Objectives**

The aims of this study were to investigate the impact of a self-paced implementation of the SYN and VIT tasks on the resulting statistical parametrical maps. Furthermore, we wanted to explore the impact of using a BL versus an ER analysis approach. Based on these objectives the examined hypothesizes were as follows:

- Results would be comparable in the self-paced paradigm with the fixed paradigm used in the original versions.
- ER analyses will show lower design efficiency, but higher specificity of activation.
- Lateralization of activation will differ between the two analysis approaches, with a higher degree of lateralization in the ER analysis.
- The time needed for the completion of the trials would differ from the previously used fixed 5 seconds.

## Materials and methods

Participants

As this study was aimed at methodological improvements, we abstained from including children (vulnerable populations according to the Declaration of Helsinki, World Medical Association, 2013). Instead, we aimed at including 20 right-handed, healthy adults [age (mean ± standard deviation): 31.7 ± 7 years, range: 21.7-43.2 years, 12 females]. The language abilities of the participants were tested by the German version of the Peabody Picture Vocabulary Test (PPVT), results reflected above-average language abilities (median value was 97.5 percentile).

Technical implementation

In the active condition of the synonyms task ($SYN_{AC}$), the participant has to determine if two visually presented words are synonyms or not. In the control condition ($SYN_{CC}$), two nonsense letter strings are to be compared and the participant has to decide whether they are identical or not. In both conditions, responses are recorded by button presses. In the active condition of the vowel identification task ($VIT_{AC}$), the participant has to recognize a pictographically presented object and match a name to it (covert picture naming) and subsequently analyze the phonological structure of this word to decide whether it contains the sound of the vowel "i" (always pronounced [i:] in German). In the control condition ($VIT_{CC}$), a pair of complex, but abstract patterns are displayed, and the participant has to decide whether the smaller one fits into the larger one "like a piece of a puzzle". Again, button presses are used to record the participant's responses. All participants completed both tasks (SYN and VIT) in the modified version.

The modification of the tasks from the previously used, pure BL design versions was done to now include aspects of a mixed BL/ER design. This was achieved by keeping the overall "blocked" task layout; for example, in the first 30 seconds, only stimuli of the control condition were displayed, while in the subsequent 30 seconds, only stimuli of the active condition were presented (and so on). However, within these blocks, the participant was able to advance stimulus presentation by a "yes" or "no" button press in each condition in the participant's right hand. Events were defined as beginning with the appearance of the stimulus and ending with a button press. The number of trials in each condition depended on the response time of the participant (the quicker the responses, the more trials could be processed in any given condition).

Data acquisition, processing and analysis

Imaging was performed on a 1.5 T whole-body MR scanner with a 12-channel head coil. An EPI-sequence was used to acquire functional series in each participant, covering the whole brain. A T1-weighted anatomical 3D-dataset and a gradient-echo B0-fieldmap were also acquired. Datasets were processed using SPM8 software, running in Matlab. The first 10 scans of each functional series (corresponding to the first block of the control condition) were rejected to allow for the stabilization of longitudinal magnetization, leaving 100 scans per series (5 blocks each of the active and the control condition). Functional images were realigned and unwarped using the individually acquired B0 fieldmap, correcting for both EPI and motion * B0 distortions. Following coregistration of the functional and the anatomical images, global signal trends were removed and functional images were smoothed with a 6 mm full width at half maximum Gaussian filter. Anatomical images were segmented using a priorless extension to the SPM segmentation algorithm as available in the VBM toolbox. Spatial normalization was achieved with the diffeomorphic anatomical registration approach using exponentiated Lie algebra (DARTEL).

First level (individual) statistical analyses were done in native space, using the General Linear Model. Each statistical analysis resulted in one parameter map, ultimately leading to 4 parameter maps per participant (two for each of the two tasks). These were then spatially normalized and entered into second level (group) random-effects analysis. To this effect, one-sample t-tests were applied on a voxel-by-voxel basis to detect typical activation patterns on the group level, separately for each task and design. Age, gender, handedness, and performance in PPVT were used as covariates of no interest as either parameter must be expected to affect the resulting activation pattern. Further, the parameter maps of the BL and ER design of each task were compared by using a paired t-test. For all analyses, significance was assumed at a voxelwise $p \leq 0.05$, FDR-corrected for multiple comparisons and an additional cluster-level FWE-correction at $p \leq 0.05$.

Assessment of lateralization

We used the LI-toolbox with default settings. We decided to specifically investigate the frontal lobe and the cerebellum. Values of $-0.2 < LI < 0.2$ were considered bilateral; values $\leq -0.2$ were considered right-dominant, while values $\geq 0.2$ were considered left-dominant. Lateralization indices (BL vs. ER design) were assessed using the two sample non-parametrical Mann–Whitney-U test, and significance was assumed at $p \leq 0.05$. The equality of the LI variances (BL vs. ER) was examined by Levene's test, and significance level was set to $p \leq 0.05$.

Design efficiency

Design efficiency was calculated for every participant and both designs. The mean number of activated voxels at a more liberal, individual threshold of $p \leq 0.001$, uncorrected, was computed and compared in left frontal and right cerebellar regions in both designs and in both tasks, using the custom-defined masks of the LI analysis. The difference was again evaluated by using the Mann-Whitney-U test, assuming significance at $p \leq 0.05$.

## Results

Group activation maps (one sample t-test)

The second-level random-effects analysis of the BL and the ER designs showed significant left inferior frontal and left posterior temporal activation clusters, as well as mainly right cerebellar activations in the active condition of both tasks, in both designs.

Comparison of block and event-related design (paired t-tests)

When comparing the block and the event-related design (BL > ER) of the SYN task, there were no significant differences in activation in key language areas. In the inverse comparison (ER > BL), the ER analysis resulted in significantly stronger activations in left inferior frontal region and in the posterior lobe of the right cerebellum.

There were no significant differences in language-related brain regions in the BL > ER comparison of the VIT. In the inverse comparison (ER > BL), a stronger activation was found in the lingual gyrus bilaterally.

Lateralization

Similar lateralization patterns were found in the BL and ER designs in both tasks. In the SYN task, frontal activation was left-dominant ($LI_{BL}$: median: 0.58, range: -0.25 to 0.84; $LI_{ER}$: median: 0.54, range: –0.25 to 0.82), while cerebellar activations showed right dominance ($LI_{BL}$: median: –0.41, range: –0.81 to 0.84; $LI_{ER}$: median: –0.44, range: -0.81 to 0.8). In the VIT, similar left frontal and right cerebellar dominance was detected (frontal: $LI_{BL}$: median: 0.56, range: –0.05 to 0.85; $LI_{ER}$: median: 0.52, range: 0.00 to 0.85; cerebellar: $LI_{BL}$: median: –0.41, range: –0.82 to 0.5; $LI_{ER}$: median: –0.4, range: –0.79 to 0.29). There was no significant difference in lateralization between task designs, in either region (all Mann-Whitney-U with $p > 0.05$). The observable variance of results was also comparable (all Levene's $p > 0.05$).

Participant performance, design efficiency and mean number of activated voxels

In both tasks, the average time required for stimulus processing ranged from 1.25 to 1.79 seconds, more than 2.5-times shorter than the fixed 5-second SOA in the original versions. Reaction times were significantly faster in the active condition (Wilcoxon rank sum test, $p < 0.01$), but the number of trials was not significantly different for either task (Mann-Whitney-U test, $p > 0.05$ for both tasks).

As expected, design efficiency was lower for the ER implementation, with a mean of 66.19 ± 5.02% for the SYN task and a mean of 65.78 ± 9.44% for the VIT task (when related to the respective BL design implementation). The mean number of voxels activated was not significantly different in the left frontal lobe or the right cerebellum between the BL and ER implementations in both tasks (Mann-Whitney-U, all $p > 0.05$).

## Discussion

Effects of adding a self-paced component

The self-paced modification of both tasks resulted in a highly specific and selective pattern of activation in key language areas, such as left inferior frontal and left posterior temporal regions, as well as right posterior cerebellar activations. This activation pattern confirms previous results (when using a fixed SOA) very nicely, demonstrating that the overall pattern does not change as a function of said modification.

The almost 2.5-fold difference between the fixed 5-second SOA used previously and the average reaction time of our participants in this self-paced modification underlines the advantage of using a self-paced paradigm, as subjects can perform at their own pace. Passive waiting harbors the danger of "mind-wandering", leading to decreased task-related activations or even more task-related deactivations.

All of this argues in favor of using a self-paced approach for participants with above-average language abilities.

On the other hand, frustration will be induced if trials are too fast for participants with below-average language abilities, likely reducing subsequent task adherence and compliance. In those participants, even though fewer stimuli may be processed as in a design with a fixed SOA, the time spent “on the task” constructively must be expected to be higher. Further, the self-paced implementation provides an effective way to monitor task performance and adherence, which is often a cause of concern in fMRI studies requiring covert responses.

Our mixed BL/ER implementation ensures that the overall time spent on each condition is inherently constant in all participants, which is an improvement over a pure self-paced, ER implementation and thus an “added bonus” of this approach.

Comparison of specificity between block (BL) and event-related (ER) design

The fact that the ER analysis found more prominent task-related activations in core language areas is surprising as such approaches have inherently lower design efficiency. However, this was not accompanied by a significant reduction in the number of activated voxels on the individual level, suggesting that this reduced design efficiency was not detrimental to sensitivity in our participants. In fact, in both the BL and ER implementations, all subjects showed suprathreshold activation in the left frontal lobe in each task irrespective of the analytical approach, illustrating that both tasks robustly elicit activation in core language regions.

Implications for pediatric fMRI studies

Successfully performing language fMRI in children is challenging on several level, moreover in a clinical setting the intersubject variability in a patient population may be increased substantially due to age or disease-related factors determining the level of cooperation. Developing tasks that can be used effectively in this setting is an important challenge, and based on our here-reported results, the self-paced implementation in combination with an ER design may offer an interesting alternative.

Lateralization

The left frontal and right cerebellar lateralization pattern was constant across both tasks and analysis methods. Although we hypothesized that the ER design may yield a higher degree of lateralization due to its higher specificity, only a tendency to provide a higher consistency (lower variability) of lateralization seemed to be present, but this difference was not statistically significant.

The consistently contralateral frontal and cerebellar activation pattern suggests that combined assessment of both frontal and cerebellar regions may increase the reliability of the verdict of "hemispheric dominance" on the individual level.

## Investigation of the brainstem with diffusion tensor tractography

### Introduction

In vivo examination of the brainstem is still a challenging field of modern neuroimaging, due to its complex anatomical structure which integrates important components of systems regulating cognitive-, motor-, and sensory functions, and consciousness. Several studies using diffusion magnetic resonance imaging have investigated the brainstem so far, but most of them relied on normal brainstem anatomy rendering the applicability of these methods very limited in pathologies causing distortion and loss of anatomical landmarks.

Behrens et al. were the first to use diffusion tensor imaging (DTI)-based probabilistic tractography to perform connectivity-based segmentation of the thalamic nuclei. Connectivity-based segmentation offers parcellation of a cortical or subcortical area without a priori information on the distribution of the connecting areas and without reliance on anatomical landmarks within the cerebral structure.

### Objectives

- To elaborate an application of connectivity-based segmentation for the identification of the most important functional subregions of the brainstem. The main functional systems in the brainstem that this work was focused on regulate cognitive-, motor-, and sensory functions and consciousness: 1, frontopontine pathways, which represent connections to the prefrontal cortex playing a decisive role in cognitive processes, 2, corticospinal and corticobulbar tracts (CST/CBT), being the efferent pathway of the primary motor cortex, 3, sensory connections involving the spinothalamic tracts and the medial lemniscus and 4, reticular formation being an important component of the ascending reticular activating system (ARAS), which controls arousal.

- To identify potential biomarkers that may be of significant help in the diagnosis and prognosis estimation of brainstem pathologies (e.g., pediatric cerebral malformations, brainstem injury due to head trauma, tumors, demyelinating disorders).

## Materials and methods

Study population

20 healthy adult participants (age range: 21.7-43.2 years, 12 females) were enrolled in the study. None of them had any previous history of neurological or psychiatric disease.

Data acquisition and preprocessing

Scanning was conducted on a 1.5 T GE Signa Excite scanner. High resolution T1-weighted scans (3D IR-FSPGR, voxel size:1 $mm^3$) and diffusion-weighted images (voxel size:2.4 $mm^3$, two repetitions, b = 1000 $s/mm^2$) in 60 independent directions and six non-diffusion weighted sets (b = 0 $s/mm^2$) were acquired.

MRI data were preprocessed using tools from the FMRIB Software Library (FSL, v5.0,; FMRIB's Diffusion Toolbox [FDT], v3.0; Oxford Centre for Functional MRI of the Brain [FMRIB], UK; www.fmrib.ox.ac.uk/fsl). Eddy current and head motion correction, followed by skull stripping, reconstruction of diffusion tensors, and estimation of diffusion parameters were done. Individual fractional anisotropy (FA) maps were registered to the MNI standard brain. Individual FA maps were mathematically summed and averaged to create a group FA map.

Selection and definition of masks

In order to identify the four brainstem subregions (frontopontine, motor and sensory pathway, reticular formation) within the pontomesencephalic portion one seed and six target masks were used. The pontomesencephalic portion of the brainstem was defined as the seed mask in this study. Target masks were the following: a coronal single-slice section of the left (1) and right (2) anterior limb of the internal capsule (ALIC) to detect frontopontine connections, an axial single-slice section of the left (3) and right (4) posterior limb of the internal capsule (PLIC) to trace the CST/CBT, and the bilateral sensory (5) and medial (6) thalamus to find the main ascending sensory pathways (medial lemniscus, spinothalamic pathways) and the reticular formation, respectively.

Probability distribution maps of the subregions and brainstem connectivity maps derived by hard segmentation at the individual and group level

Probabilistic tractography was performed with default settings of the FMRIB Diffusion Toolbox to generate probability distribution maps (PDM) of the six subregions (left and right frontopontine, left and right motor subregions, sensory pathways and reticular formation) at the individual and group level. Then connectivity maps (CM) were created from the PDMs by hard segmentation for all participants individually and a group CM as well. The CM consisted of six connectivity defined regions (CDR), corresponding to the location of the PDMs. To eliminate low-probability connections, eight different threshold levels (1%, 5%, 10%, 15%, 20%, 25%, 35% and 50%) were tested from which the 25% seemed to be the most anatomically plausible.

Quantitative assessment of the reproducibility of the connectivity-based brainstem segmentation

The center of gravity of the connectivity values ($CoG_{conn}$) in each axial slice of the PDMs was chosen to quantitatively evaluate the consistency of the results of the connectivity−based brainstem segmentation across our subjects. To exclude collateral connections, we applied masking of the PDMs. For this purpose, a group probability mask was created for each subregion. To this end, from individual CMs in standard space the six CDRs were separated. Then the same CDRs were binarized and summed to create a group probability mask of the given CDR. All group probability masks were thresholded to exclude remaining collaterals.

We used the group PDM as a reference for the measurement of the distances of the individual $CoG_{conn}$ locations. The unthresholded group and individual PDMs in standard space were masked with the corresponding group probability mask. In the next step, we calculated the distance of the $CoG_{conn}$ of the group and the corresponding individual PDMs in each axial slice from z = - 10 to z = - 48.

Comparison of segmentation results to microscopic anatomy and anatomical reference material

Anatomical structures were located within the group connectivity map by an experienced neuroanatomist and the help of the corresponding sections of the Nieuwenhuys atlas. The latter were overlaid on corresponding histological sections of the brainstem and matching sections of the MNI152 1 mm standard brain. The anatomical structures identified were the followings: frontopontine tract, corticospinal and occipitoparietotemporopontine tract, medial lemniscus, spinothalamic tract, central tegmental tract, superior cerebellar peduncle, dorsal longitudinal fasciculus.

Assessment of subregion-specific quantitative measures (connectivity, FA)

We investigated the relationship between the connectivity values of the PDMs and the corresponding FA values, and whether characteristic subregion-specific changes can be detected in the connectivity and FA values along the rostrocaudal axis of the brainstem.

First, we examined the extent of overlap between the maximum connectivity and the maximum FA voxels in each axial slice of the masked individual PDMs.

Using the masked individual PDMs and FA maps registered to standard space, the individual and group mean connectivity and mean FA values were calculated for each axial slice of each subregion. We aimed at evaluating the relationship of these group mean connectivity and FA values with the rostrocaudal position (z coordinates) of the given axial slice in standard space by performing Spearman's rank correlation and Pearson's correlation. Significance was assumed at $p < 0.05$.

Two illustrative cases of severe traumatic brain injury

Two patients with severe traumatic brain injury (TBI) and similar initial clinical presentation but different outcomes (improvement to self-care vs. persistent vegetative state) were selected to test clinical applicability of the connectivity-based brainstem segmentation. Both of them underwent the same diffusion MRI protocol and MRI data processing as described above. The mean connectivity and mean FA values for each axial slice of each subregion were also calculated.

**Results**

Segmentation pattern on the group level

The medial portion of the cerebral peduncles was dominantly connected to the left and right ALIC (frontopontine CDR). This CDR could be followed downwards until the mid-pons. The middle and lateral portions of the cerebral peduncles and both sides of the pontine basis dominantly connected to the left and right PLIC (motor CDR). The dorsolateral part of the mesencephalon and the border of the pontine tegmentum and basis were dominantly connected to the sensory thalamus (sensory CDR). The mesencephalic and pontine tegmentum were dominantly connected to the medial thalamus (reticular CDR).

Segmentation pattern on the individual level

The individual CMs were very similar to the group CM in most subjects, and all four subregions were successfully identified in 13 out of the 20 healthy subjects (65%). The distance between the individual and group $CoG_{conn}$ locations was dominantly in the range of one voxel in MNI152 2 mm standard space (91.74% of the calculated distances were ≤ 2 mm) reflecting good consistency of the individual results of the segmentation.

Comparison of segmentation results to microscopic anatomy and anatomical reference material

Overlaying the corresponding sections of the Nieuwenhuys atlas on the histological sections and the MNI152 1 mm standard brain MR images, the group CM was in good overall visual concordance concerning the spatial distribution of the identified CDRs with the pathways displayed in the atlas and determined on the histological sections. In case of the sensory CDR, in the pontine region part of the medial lemniscus was identified in the motor CDR.

Assessment of subregion-specific quantitative measures (connectivity, FA)

The maximum connectivity and maximum FA voxels in each axial slice of the individual PDMs overlapped in 5.5-33.5% of the cases, with the highest rate of overlap in the motor subregion and the lowest rate of overlap in the reticular subregion. The distance between the maximum connectivity and the maximum FA voxels in each axial slice of the PDMs was ≤ 2 mm in 51.7%, > 2 mm and ≤ 4 mm in 28%, and above 4 mm in 20.3 % of the cases.

The investigation of the rostrocaudal connectivity and FA value changes in the detected subregions revealed a characteristic location-dependent pattern along the longitudinal axis of the brainstem in all subregions. The relationship of the mean connectivity values to their rostrocaudal position (represented by the z coordinate of the given axial slice) was proven to be monotonous in all subregions. The relationship of the mean FA values to their rostrocaudal position was monotonous in the frontopontine subregion, and not only monotonous but also linear in the motor subregion. In the sensory and reticular subregions mean FA values were shown to be in a nearly parabolic relationship with their rostrocaudal position. That is, the highest mean FA values in the sensory and reticular subregions were observed in the upper pons, and lower values were found in the mesencephalon and the lower pons.

Two illustrative cases of severe TBI

On both the FLAIR and SWI images of Patient 1 (who improved from traumatic coma to self-care), bilateral diffuse axonal injury was seen in the dorsal pontomesencephalic region, whereas in the case of Patient 2 (whose outcome was persistent vegetative state), this region appeared to be intact on these sequences. In case of Patient 1, the connectivity-based brainstem segmentation showed diminished but existing connectivity in all examined subregions compared to the healthy subjects. However, in the case of Patient 2, the connectivity-based brainstem segmentation revealed a more widespread damage of the brainstem with left-sided predominance. In the mesencephalon, all subregions could be detected with diminished connectivity, but no connectivity to the medial thalamus could be identified in the pons, nicely explaining the clinical outcome.

## Discussion

Correspondence with the known anatomy and reproducibility of the segmentation

The location of the CDRs of the group CM showed an overall good concordance with the anatomy identified on the histological sections and a neuroanatomy atlas and also corresponded to the results of former DTI studies, providing a good basis for quantitative analyses of the examined brainstem subregions.

On the individual level, all four subregions were successfully identified by the hard segmentation in 13 out of 20 subjects (65%). In the remaining 7 subjects the connectivity pattern of the different PDMs was similar to that of the other 13 subjects.

In these 7 subjects the small differences between the connectivity values within the neighboring reticular and sensory PDMs could mislead the hard segmentation (as it relies on the ”winner takes it all” principle), and precluded the detection of one of these subregions.

The distance between the group and individual $CoG_{conn}$ values reflected good reproducibility of the connectivity measures within the identified subregions.

Subregion-specific quantitative measures (connectivity, FA)

Our aim was to assess changes in the connectivity and FA values in the identified subregions along the rostrocaudal axis of the brainstem. The similar shape of the rostrocaudal FA profiles of the subregions within the pontomesencephalic region observed in our study and in previous works further supports the validity of our results. In case of the frontopontine subregions the mean connectivity and FA values showed a monotonous decrement in caudal direction, while in the motor subregions the curve of the mean FA values followed not only a monotonous, but a linear course. The decrease of the FA values in the caudal direction may be due to the spreading of the frontopontine fibers as they terminate on the pontine nuclei, and the interposition of the transverse pontine fibers traversing the corticospinal tract in the pons. In the reticular and sensory subregions a relative plateau of the mean connectivity values was observed in the pons, while FA profiles followed a U-shape. These characteristic changes may indicate structural differences between these subregions, and therefore may serve as potential biomarkers in the investigation of brainstem pathologies.

Skeletonizing white matter pathways according to their maximum FA voxels is a popular way to investigate microstructural white matter differences between groups of subjects. Comparing the location of the maximum FA voxel to the maximum connectivity voxel within each axial slice of the subregions revealed a low percentage of overlap, being the highest in the motor subregion, and the largest distances were observed in the reticular subregion. FA measurements are based on local diffusion parameters, while connectivity relies on more global diffusion information. The high ratio of divergence between the highest FA and connectivity values raises the possibility that connectivity may be a more sensitive parameter for the investigation of the compact structure of the brainstem, especially the reticular formation.

Potential clinical applicability

Our results obtained in 20 healthy subjects may provide reference for future studies investigating various pediatric and adult patient populations. The connectivity-based identification of the pontomesencephalic reticular formation may serve as a basis for quantitative studies using diffusion MRI-related biomarkers to assess the anatomical components of arousal in patients with TBI. Former studies suggested that traumatic coma may be a result of white matter injury in the ARAS. In the presented two cases of severe TBI, the results of the connectivity-based brainstem segmentation predicted the final clinical outcome as opposed to the conventional MRI sequences and the FA values, therefore this method may provide useful additional information for the prediction of long-term outcome in patients with severe TBI. Further candidate pathologies may be space-occupying lesions (brainstem tumors), pediatric cerebral malformations affecting the pontocerebellar region, brainstem stroke, and disorders of myelinisation affecting the brainstem.

## Conclusions

In the past three decades, functional and diffusion magnetic resonance imaging have emerged from purely research methods to clinical tools, increasingly playing a role as a significant add-on in the diagnosis and therapy of central nervous system diseases. During my PhD work I applied these state-of-the-art imaging techniques to develop potential non-invasive clinical tools in the diagnosis and therapy of several diseases of the central nervous system.

The self-paced implementation of the SYN and VIT fMRI tasks in a mixed BL/ER fashion seems to be a well-applicable alternative to a pure block-design approach, making both tasks more suitable for use in participants with both above- and below–average language abilities. Moreover, our results confirmed that the ER analysis of the paradigm provides more specific detection of the productive language network. Despite the lower design efficiency, individual sensitivity to activation was not affected. We therefore suggest that these modifications should be considered when aiming to investigate language functions in a presurgical setting in particular.

Following the principles of the connectivity-based thalamus segmentation elaborated by Behrens *et al.* in 2003, we successfully segmented and identified four main functional subregions (cognitive, motor, sensory and reticular formation) in the brainstem, and found potential diffusion-tensor imaging-based quantitative biomarkers that were successfully tested in the diagnostic workup of TBI.

Both new methods warrant further research to test their applicability in various disease states and patient populations.


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