Project Description – Project Proposals

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Vascular resistance and resilience in ALS – an ultrahigh-resolution 7T MRI study of the motor cortex

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Project Description

Sections 1-4 must not exceed 15 pages in total.

1 Starting Point

1.1 State of the art and preliminary work

Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disorder. Particular in the motor cortex, the disease's hallmark inclusion body pathology, i.e. abnormally ubiquitinated and phosphorylated aggregates of 43 kDa transactive response DNA-binding protein (pTDP-43) in degenerating motoneurons, is already found at the beginning of the disorder (Braak et al., 2013). Clinically, motor cortical motoneuron degeneration first affects one body part of the motor homunculus of one hemisphere, reflected by focality of initial motor phenotypes classified as bulbar or left or right upper or lower limb onset (Ravits et al., 2007). Motor cortical motoneuron degeneration then seems to have a preferential and sequential direction of propagation from lateral to medial body parts (bulbar, upper limb, lower limb) of the ipsilateral hemisphere, and afterwards to the contralateral hemisphere; clinical symptoms accordingly rather evolve from rostral to caudal regions (Ravits et al., 2007).
Afterwards, pTDP-43 pathology further propagates sequentially to the prefrontal, postcentral and temporal neocortex, the basal ganglia and the medial temporal lobe (MTL) (Brettschneider et al., 2013). Neuronal pTDP-43 aggregation goes along with a loss of the protein's physiological functions, i.e. such as DNA damage repair and axonal transport, overall fostering neurodegeneration. Motoneurons have a high-energy metabolism and demand, and are consequently prone to oxidative stress, mitochondrial dysfunction, hyperexcitability and, thus, glutamate-mediated excitoxicity, considered upstream events promoting motor cortical pTDP-43 aggregation and propagation, and, thus ALS initiation and clinical manifestation (please see (Asakawa et al., 2021) for review).

ALS is rare, but in the next 20 years there will be a global increase of affected patients by 69%, mainly due to ageing of the population among developing nations (Arthur et al., 2016). Only around 5-10% suffer from hereditary (familial) ALS, while the underlying risk for or resistance against the remainder of 90-95% of sporadic disease is still poorly understood (Taylor et al., 2016). The same holds true for disease course, with only few clinical variables considered predictors of more rapid decline (e.g. weight loss before ALS diagnosis, bulbar onset, diagnostic delay, cognitive impairment (Paganoni et al., 2011; Westeneng et al., 2018)). Accordingly, despite the majority of ALS patients dying within 3 to 5 years of onset, the disease is highly heterogeneous and the length of survival is markedly variable in the individual. Indeed, some patients have a very long survival of up to 10 years from diagnosis, and in some the disease progression seems to cease or even to reverse (Bedlack et al., 2016; Pupillo et al., 2014). Consequently, there must be factors and mechanisms of "resistance" (i.e. tolerance against pathology ["avoiding"]) and "resilience" (i.e. tolerance against the impact of pathology ["coping"]) in ALS with an imperative need for elucidation to open the door for new preventive and therapeutic strategies in this incurable neurodegenerative disorder (Arenaza-Urquijo and Vemuri, 2018; McCombe et al., 2020).

The concept of resistance and resilience has gained a lot of interest in neurodegeneration, and corresponding research has thus far focused on (preclinical) Alzheimer's disease identifying

lifestyle or behavioral modifications (e.g. intellectual enrichment, physical activity) to play an important role (Arenaza-Urquijo and Vemuri, 2018; Schreiber et al., 2016).
Just recently, in aging, systemic vascular health, defined through a sum score of low cardiovascular risk and disease, has additionally proven to maintain normal cognition through preserved neuronal metabolism in corresponding "resilience signature" brain regions (Arenaza-Urquijo et al., 2019).

We lately defined a specific measure of vascular health that provides "vascular resistance" and "vascular resilience" to the brain, namely patterns of vascular supply. Applying 7 Tesla (T) *in vivo* time-of-flight magnetic resonance angiography (ToF MRA) to the aging MTL, we discovered that the vascularization through the terminal small blood vessels (average luminal diameter of 500 µm) from two arteries ("double supply") instead from one artery only ("single supply") related to better preserved MTL gray matter volume and memory function (Perosa et al., 2020b; Perosa et al., 2020a).
Interestingly, vascular resistance and vascular resilience provided by patterns of MTL double supply transferred to brain regions beyond the MTL, in terms of better sustained whole-brain gray matter volume and overall cognitive performance (Perosa et al., 2020b; Vockert et al., 2021).We speculated, that in case of double supply vascular resistance and vascular resilience was provided by overlapping perfusion territories of the feeding arteries supposed to be indicative of higher small blood vessel density and/or better (sustained) small blood vessel function, i.e. reactivity, ensuring optimal neuronal oxygen and energy supply as well as the removal of toxic metabolites from the brain.

Variable individual patterns of vascular supply are not only found in the hippocampus, but are evident throughout the whole brain, including the human motor cortex.
One postmortem study in 20 adults reported supply of the medial motor cortex by terminal cortical small vessel branches (average luminal diameter of 650 µm) of two arteries (pericallosal and callosomarginal artery) that derive from the anterior cerebral artery (ACA). While the lateral motor cortex is supplied by the branches of three arteries (precentral/central and postcentral group) from the middle cerebral artery (MCA) (Ugur et al., 2005).
Prevalence of double supply from ACA (pericallosal plus callosomarginal artery) vs. single supply (pericallosal or callosomarginal artery) was 30% vs. 70% (30% pericallosal artery dominance; 40% callosomarginal artery dominance). For MCA, double supply (precentral/central plus postcentral group) vs. single supply (precentral/central without postcentral group) was found in 70% vs. 30% of the cases (Ugur et al., 2005).

As a proof-of-concept, we determined for 21 healthy young volunteers the ACA and MCA vascular supply patterns of the motor cortex in vivo using 7T MRI (collected retrospectively from data repository, 450 µm Magnetization Prepared Rapid Gradient Echo (MPRAGE) images). **Figure 1** demonstrates ACA double vs. single supply in two exemplary young cases.
In vivo prevalence of ACA and MCA double vs. single supply was in concordance with postmortem findings (ACA: 33 % double vs. 67 % single supply; MCA: 67 % double vs. 33 % single supply).
Although this is only an early indicator, the similar frequencies hint at a stability of the supply patterns through the lifespan.

In ALS, vascular health seems to be of pivotal importance as well for protection from disease and slowing of the disease course (Kuraszkiewicz et al., 2020), which is supported by several clinical observations. First, ALS patients suffer significantly more often from cerebrovascular disease compared to controls; secondly, ALS patients with comorbid cardiovascular risk and disease show more rapid disease course than ALS patients without; and, further, ALS patients display small vessel abnormalities affecting the brain, retina, muscle and skin (Abdelhak et al., 2018; Buckley and Bossen, 2013; Diekmann et al., 2020; Kolde et al., 1996; Mandrioli et al., 2018; Saul et al., 2020). We hypothesize, that vascular health interacts with the disease initiation and clinical manifestation, and, in that, we are specifically convinced that vascular supply as a certain measure of vascular health contributes to vascular resistance and vascular resilience in ALS.

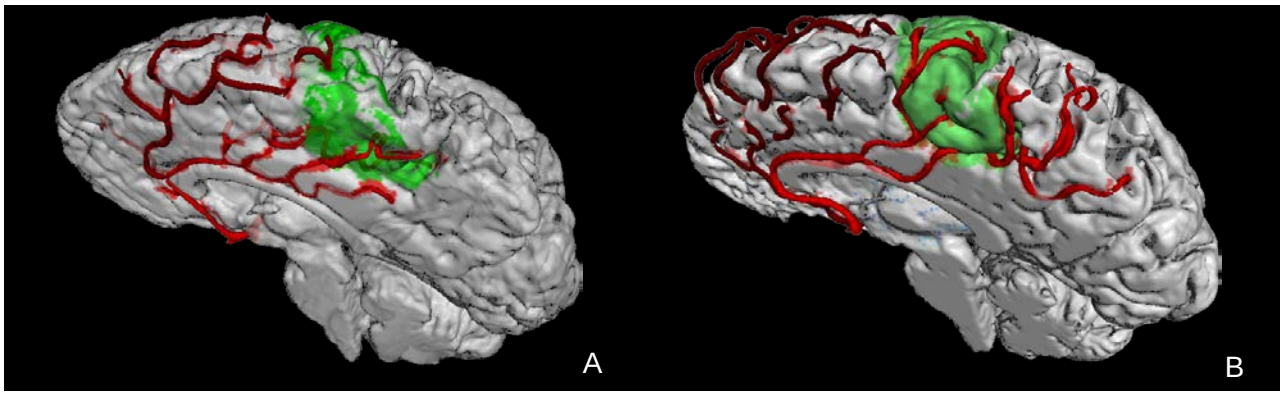

Figure 1. Vascular supply patterns of the motor cortex through the anterior cerebral artery in healthy young controls. In **A** shared motor cortical supply by branches of the pericallosal artery (light red) and callosomarginal artery (dark red) is shown ("double supply"). **B** displays a dominant motor cortical supply by branches of the pericallosal artery (light red) ("single supply"). The motor cortex is colored green. Volunteers received 7T MPRAGE scans; vessels were manually de-lineated; the motor cortex was segmented using Freesurfer.

The following mechanistic underpinnings support this hypothesis:
The expression of the vascular endothelial growth factor (VEGF) is reduced in ALS rodent models and patients (Brockington et al., 2006; Lambrechts et al., 2003). VEGF exerts trophic and neuroprotective effects on motoneurons (Oosthuyse et al., 2001). One hypothesis to explain these protective VEGF effects is that it promotes a "vascular niche": after secretion from motoneurons and endothelial blood vessel cells, VEGF fosters vascular endothelial cell and, thus, small vessel formation. This guarantees an optimal oxygen and energy supply and toxic metabolite removal, protecting motoneurons from oxidative stress and glutamate-mediated excitotoxicity, and, thus, from pTDP-43-mediated neurodegeneration (please see (Silva-Hucha et al., 2021) for review). Accordingly, experimental VEGF reduction has been related to accelerated motoneuron degeneration and disease onset, while experimental VEGF overexpression has been shown to cause the opposite, i.e. prolonged motoneuron survival and delayed disease onset (Azzouz et al., 2004; Lambrechts et al., 2003; Storkebaum et al., 2005).
Further, small vessel pericyte coverage and integrity is reduced in ALS models and patients (Garbuzova-Davis et al., 2012; Winkler et al., 2013). Pericytes promote small vessel wall stability, and regulate cerebral blood flow (CBF) and cerebrovascular reactivity (CVR), and toxic metabolite removal (Rustenhoven et al., 2017). In ALS, pericyte deficiency goes along with a reduction of the total small vessel length, collapsed small vessel lumens and aberrant angiogenesis (Sasaki, 2015; Yamadera et al., 2015; Zhong et al., 2008). Subsequent CBF/CVR reduction fosters insufficient oxygen and energy supply, and hinders toxic metabolite removal, which precedes and promotes motoneuron degeneration (Zhong et al., 2008). Hence, experimental prevention of pericyte loss slowed motoneuron degeneration and ALS disease course (Coatti et al., 2017).

We here aim to investigate vascular supply of the motor cortex applying 7T *in vivo* MRI as one driving factor for the large variance of pathology and motor phenotypes in ALS, hence contributing to resistance and resilience in motor neuron disease. We hypothesize that regions with a double supply are more resistant to ALS pathology (i.e. motoneuron loss and corresponding cortical atrophy (Chen et al., 2018)) than regions with a single supply, because the proximity to multiple feeding arteries is associated with a protective overlap of perfusion territories ensuring optimal oxygen and energy supply. We expect that less ALS pathology mediates the resilience effect of double supply, i.e. overlapping perfusion territories, on less severe global and body part specific motor function and its progression in ALS (see **Figure 2A & 2B**).

To assess the feeding arteries of interest and even their very small higher order branches *in vivo*, the sub-millimeter imaging capabilities of 7T MRI combined with prospective motion correction (Stucht et al., 2015) promise the highest non-invasive angiographic quality. Besides the increased signal-to-noise ratio, prolonged T1-relaxation times at 7T enhance the contrast-to-noise ratio of small arteries in ToF MRA and MPRAGE sequences. Motion correction allows to exploit these effects beyond the biological resolution limit (Stucht et al., 2015).

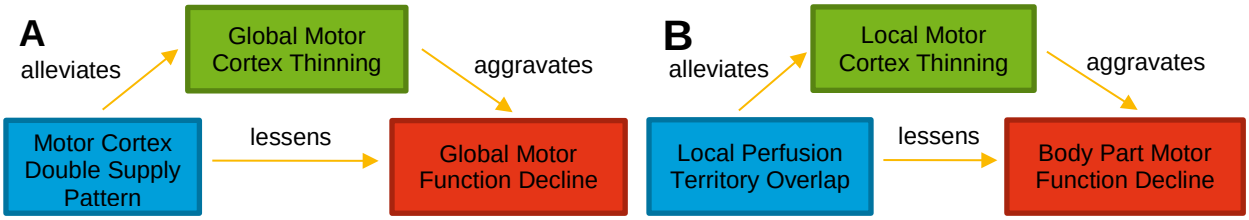

Figure 2. Proposed mediation model of the relationship between vascularization, motor cortex atrophy and motor function. **A** Global assessment of vascularization via the categorization into motor cortex vascular supply patterns and their influence on current global motor function and its decline potentially mediated by generalized thinning/atrophy of the motor cortex. **B** Investigation of the artery-specific vessel distance mapping (perfusion-territory) information for the area representing the respective body part in the motor cortex homunculus with local cortical thinning as a mediator for current and longitudinal body part specific motor function.

With decreasing diameter of e.g. the terminal branches supplying a certain brain region, the perfusion territory of an individual small artery becomes more spatially specific (Feekes et al., 2005; Hendrikse et al., 2010). For higher-order branches, hence arteries with small diameter, the perfusion territory becomes roughly spherical and the summation of these spheroidal-shaped territories approximates the perfusion territory of the feeding artery (Feekes et al., 2005). Thus, the supply territory of a feeding artery is characterized by the distribution of and distance from its branches.

This data-driven approximation of arterial supply territories can be achieved by vessel distance mapping (VDM). VDM is a technique recently proposed by us (Mattern and Speck, 2020) and funded by the DFG (DFG-NAMT9 MA 9235/1-1). Briefly, VDM computes the Euclidian distance for each non-vessel voxel to its closest small artery (diameter < 300 µm) in high-resolution MR images (detailed description in work packages).

To investigate double and single motor cortex supply patterns, VDM will be assessed artery-specific. To that end, the distance maps will be computed for all feeding arteries originating from the ACA and MCA individually (see **Figure 3**). Therefore, the distance of every voxel to each feeding artery is determined. If a voxel is in close proximity to two feeding arteries, the artery-specific distances will be low. The root-mean-square (RMS) of the artery-specific VDMs is proposed to combine the distances into a single map. From the RMS VDM, overlapping supply territories can be approximated by identifying regions associated with low RMS VDM values.

The high-resolution data enables us to approximate the perfusion territories of each body part of the motor cortex homunculus individually using artery-specific VDM. Again, the rationale is that areas with low RMS distances can be classified as double supply territories, which we hypothesize to be more resistant against ALS pathology. Artery-specific VDM could predict resilience, i.e. the degree of motor function and its longitudinal loss for each body part separately, which – again – might be mediated by the degree of pathology of the corresponding area of the motor cortex (see **Figure 2B**).

In summary, for the classification, it is sufficient to visually depict the feeding arteries originating from the ACA and MCA. Hence, this technique could be used at clinically available field strengths (translation into clinical practice). Artery-specific VDM extents the binary classification to an intra-cortical and body part level and correlates perfusion territories, local vascular reactivity, local cortical atrophy and focal motor function impairment in an explorative manner. Thus, the proposed methods represent a novel use-case of ultra-high field MRI and allow subject-specific predictions about ALS pathology and longitudinal motor function failure, which could improve disease management in the future.

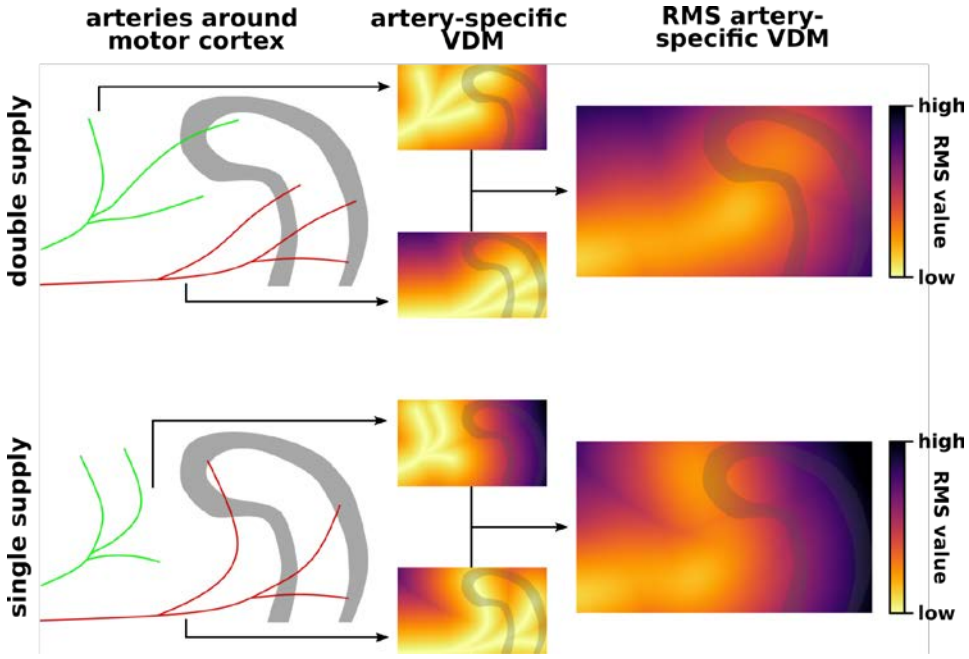

Figure 3: Approximation of supply territories by root-mean-square (RMS) artery-specific vessel distance mapping (VDM). VDM is computed for each artery respectively. The RMS of these VDMs identifies areas with low distances to both supply territories. A double supply pattern is associated with low distance in the motor cortex, hence, has a high protective overlap of supply territories. For a single supply pattern, the motor cortex is supplied by a single feed artery with no or only minimal protective overlap.

Specifically, we formulate the following hypotheses:

Hypothesis 1: There is a failure of vascular resistance in ALS.

1a Compared to controls in ALS motor cortex double supply through ACA and/or MCA is less common.

2a The observed supply patterns are determined and remain preserved during the disease course.

Hypothesis 2: There is a current failure of vascular resilience in ALS.

2a Single supply of ACA/MCA territories relates to worse current global motor function, which is mediated by lower CVR and more severe atrophy of the whole motor cortex.

2b High artery-specific distances, i.e. little overlap of perfusion territories of the feeding arteries assessed through VDM, relate to worse current body part specific motor function, which is mediated by lower CVR and more severe atrophy of the local body part of the motor cortex.

Hypothesis 3: There is a longitudinal failure of vascular resilience in ALS.

3a Single supply of ACA/MCA territories relates to more rapidly deteriorating longitudinal global motor function, which is mediated by faster progression of global motor cortex CVR reduction and atrophy.

3b High artery-specific distances, i.e. little overlap of perfusion territories of the feeding arteries assessed through VDM, relate to faster deterioration of body part specific motor function, which is mediated by faster progression of local motor cortex CVR reduction and atrophy.

In case, we will discover vascular resistance and vascular resilience in ALS through vascular supply patterns of the motor cortex, this will open up completely new avenues for trials seeking to find innovative biomarkers to monitor the success of preventive/therapeutic strategies against disease initiation and clinical manifestation. Vascular patterns could in that serve as a measurable marker that mediate the effect of new interventions promoting e.g. cerebral small

vessel plasticity, and thus sufficient oxygen and energy supply, on motor cortex structure and function. Further, the discovery of vascular resistance and vascular resilience in ALS will lead to new prevention and therapy strategies themselves aiming at modifying existing vascularisation profiles into desired profiles that benefit at-risk (e.g. relatives of patients with familial ALS) or clinical populations. These strategies could in that focus on (i) improving the autoregulatory properties (i.e. CVR) of the existing vascular profile and/or (ii) inducing neovascularization and thereby alter the existing vascular profile itself. These achievements could be reached by interventions that act through the improvement of endothelial/pericyte function, enhanced vasodilatation and reduced vessel stiffness.

1.2 Project-related publications

Sections 1.2.1 and 1.2.2 together must not exceed 10 publications; please number them consecutively.

1.2.1 Articles published by outlets with scientific quality assurance, book publications, and works accepted for publication but not yet published.

1. Acosta-Cabronero, J., Machts, J., **Schreiber, S.**, Abdulla, S., Kollewe, K., Petri, S., Spotorno, N., Kaufmann, J., Heinze, H.-J., Dengler, R., Vielhaber, S., Nestor, P.J., 2018. Quantitative Susceptibility MRI to Detect Brain Iron in Amyotrophic Lateral Sclerosis. Radiology 289 (1), 195–203.
2. Cardenas-Blanco, A., Machts, J., Acosta-Cabronero, J., Kaufmann, J., Abdulla, S., Kollewe, K., Petri, S., **Schreiber, S.**, Heinze, H.J., Dengler, R., Vielhaber, S., Nestor, P.J., 2016. Structural and diffusion imaging versus clinical assessment to monitor amyotrophic lateral sclerosis. Neuroimage Clinical 11, 408–414.
3. **Mattern, H.**. Sciarra, A., Godenschweger, F., Stucht, D., Lüsebrink, F., Rose, G., **Speck, O.**, 2018. Prospective motion correction enables highest resolution time-of-flight angiography at 7T. Magnetic Resonance in Medicine 80 (1), 248–258.
4. Lüsebrink, F., Sciarra, A., **Mattern, H.**, Yakupov, R., **Speck, O.**, 2017. T1-weighted in vivo human whole brain MRI dataset with an ultrahigh isotropic resolution of 250 µm. Scientific Data 4, 170032.
5. Stucht, D., Danishad, K.A., Schulze, P., Godenschweger, F., Zaitsev, M., **Speck, O**., 2015. Highest resolution in vivo human brain MRI using prospective motion correction. PLoS ONE 10 (7), e0133921.
6. Perosa, V., Priester, A., Ziegler, G., Cardenas-Blanco, A., Dobisch, L., Spallazzi, M., Assmann, A., Maass, A., **Speck, O.**, Oltmer, J., Heinze, H.-J., **Schreiber, S.***, Düzel, E.*, 2020. Hippocampal vascular reserve associated with cognitive performance and hippocampal volume. Brain 143 (2), 622–634.
7. **Schreiber S.**, Northall A., Weber M., Vielhaber S., Kuehn E., 2021 Topographical layer imaging as a tool to track neurodegenerative disease spread in M1. Nat Rev Neurosci; 22; 68–69
8. **Schreiber, S.**, Vogel, J., Schwimmer, H.D., Marks, S.M., Schreiber, F., Jagust, W., 2016. Impact of lifestyle dimensions on brain pathology and cognition. Neurobiology of Aging, 40, pp. 164-172.
9. **Schreiber, S.**, Spotorno, N., Schreiber, F., Acosta-Cabronero, J., Kaufmann, J., Machts, J., Debska-Vielhaber, G., Garz, C., Bittner, D., Hensiek, N., Dengler, R., Petri, S., Nestor, P.J., Vielhaber, S., 2018. Significance of CSF NfL and tau in ALS. Journal of Neurology 265 (11), 2633–2645.
10. Vockert N., Perosa V., Ziegler G., Schreiber F., Priester A., Spallazzi M., Aruci M., **Mattern H.**, Düzel E., **Schreiber S.**, Maass A., 2021. Hippocampal vascularization patterns exert local and distant effects on brain structure but not vascular pathology in old age. Brain Comm 2021; fcab127

1.2.2 Other publications, both peer-reviewed and non-peer-reviewed

None.

1.2.3 Patents

1.2.3.1 Pending

None.

1.2.3.2 Issued

None.

2 Objectives and work programme

2.1 Anticipated total duration of the project

2 years.

2.2 Objectives

The main goal is to identify the role of vascular supply patterns of the human motor cortex in resistance and resilience against ALS pathology. To that end the following objectives need to be addressed:

- To establish vascular supply patterns and function of the human motor cortex in ALS and controls applying *in vivo* 7T ultra-high field ToF MRA, MPRAGE and fMRI-based CVR estimates.
- To establish vessel distance mapping (VDM) as a proxy of the overlap of perfusion territories of the feeding arteries in the human motor cortex in ALS and controls
- To investigate the impact of vascular supply on current and longitudinal global and local motor cortex CVR and atrophy and how this mediates current and longitudinal global and body part specific motor function in ALS

2.3 Work programme including proposed research methods

For each applicant

- **WP1: Recruitment and clinical testing (clinical scientist)**

  Based on a power analysis (see below), we will need to include 20 ALS patients, that will be recruited from the specialist neuromuscular outpatient clinic of the Department of Neurology, Magdeburg. Building on a pool of local recently diagnosed ALS patients, recruiting for additional patients will be intensified to extend beyond local patients to the referred patients from cooperating departments in Hannover and Rostock. All patients will undergo genetic testing to exclude familial ALS, brain/spinal cord imaging at 1.5T MRI to exclude concurrent structural diseases, and vascular ultrasound to exclude stenoses/occlusions of the large cerebral arteries. At baseline, patients will undergo 7T MRI, motor function and neuropsychological testing (see below); motor function testing will be repeated at 3, 6, 9 and 12 months longitudinal follow-up. At 12 months the patients will additionally receive a follow-up 7T MRI scan.

  To reduce heterogeneity and to control for characteristics predicting more rapid decline (see 1.1), at baseline, all ALS patients need to fulfill the following inclusion criteria:

  - Age at baseline 50-70 years
  - Sporadic ALS
  - Classic ALS
  - Probable or definite ALS according to the Awaji criteria (Carvalho et al., 2008; Carvalho and Swash, 2009)
  - Limb-onset motor phenotype
  - No cognitive / behavioral impairment
  - No bulbar / respiratory impairment
  - No loss of at least 2 body mass index (BMI) units during the 6 months preceding diagnosis
  - Timespan between symptom onset and 7T MRI at most 12 months
  - Timespan between diagnosis and 7T MRI at most 3 months
  - Riluzole treatment
  - No history of cerebrovascular disease

  Motor function testing at baseline and at each follow-up will comprise assessment of global and body part specific motor function.

  - Assessment of global motor function:
    - Revised ALS functional rating scale (ALSFRS-R) total

- Assessment of body part specific motor function:
  - Bulbar: Center for Neurologic Study Bulbar Function Scale (CNS-BFS), frequency of lateral tongue movements
  - Upper limb: Purdue Pegboard, Grooved Pegboard, O'Conner Dexterity tests (left and right), hand strength measurements (hand held dynamometer)
  - Lower limb: Six-minutes-walking-test

At baseline only, cognitive and behavioral impairment will be assessed, applying the Edinburgh Cognitive and Behavioral ALS Screen (ECAS).

Twenty age- and sex-matched controls will be used in the study, as well as five healthy individuals, necessary for initial sequence testing. These will be recruited from a pool of volunteers established at the German Center for Neurodegenerative Diseases (DZNE) and the Department of Neurology (Perosa et al., 2020b). Controls will undergo one 7T MRI only.
The clinical scientist will screen and recruit the patients, accompany and supervise all participants during the scan sessions and carry out functional tests with the patients.

Power analysis:
The intended analyses focus on the evaluation of functional assessments, as well as MRI-based evaluation of local cortical thicknesses. In order to derive the necessary cohort size for this study we carried out power analyses for the available readouts based on available data from the currently existing cohort.
ALS disease progression shows a high inter-individual heterogeneity, especially in the early stage of the disease. Steinbach et al. suggest an indicator for disease aggressiveness with the D50 model, by tracking patients' loss of function during the disease course in a sigmoidal trajectory of the ALSFRS-R (Steinbach et al., 2020). The time D50 in this model represents the span from disease onset without impairment (ALSFRS-R 48) to a functionality loss of 50% at ALSFRS-R 24. To calculate necessary cohort sizes for this study with a focus on limb-onset ALS only, the aggressiveness of loss of limb-function will consequently be approximated by a derived metric, with a limb motor D50 (lmD50) as the number of months for a 50% decline of the sum of fine and gross motor sub-scores of the ALSFRS-R down from 24 to 12 (for illustration please see two exemplary cases in **Figure 4a**). We analyzed the ALSFRS-R trajectories of 164 patients from the Magdeburg ALS database that match the planned inclusion criteria. The patients' lmD50 exhibit a distribution with a wide range, skewed towards shorter times (median lmD50 27.0 months, **Figure 4b**). Only ca. 20% of the patients, those suffering from the most aggressive form of the disease, will experience their lmD50 during the 12 months follow-up period. Preliminary results indicate that for patients with medium and larger lmD50, the extrapolation from the follow-up examinations obtained during the 12 months project duration will be sufficient to distinguish the short, medium and longer lmD50 groups, as lmD50 predictions based the values from the first 12 months are highly correlated with those calculated for the whole duration of the disease (ρ=0.91, p>0.001, **Figure 4c**).

Based on preliminary data of younger participants (please see above), statistical independence of the ACA (double 33%, single 67%) and MCA (double 67%, single 33%) patterns can be assumed (X^2-test p = 0.63). Therefore, at least 22% of the patients are expected to possess single supply constellations in both arteries (Group S). Following the hypothesis that single supply of ACA/MCA territories relates to worse current and longitudinal motor function, we assume that these 22% of the patients with the most aggressive motor function decline and shorter lmD50 (mean [SD] 11.2 [3.6]) are those

that possess a single supply pattern. In contrast, the 22% with putative double supply patterns in both arteries (Group D) are expected to show the slowest motor function decline with long lmD50 (mean 65.2 [12.1]). To test the group differences under the unequal variances with a one-sided significance level of 0.05 and a power of 80% requires group sizes of at least 4 individuals per group. Considering the expected prevalence of the supply patterns, a total group size of 19 (4/0.22=18.2) ALS patients is deemed necessary for the study.

Planned clinical tests, i.e. body part specific motor function, will be able elucidate the independent effects of the two arteries' supply patterns and will allow detecting more subtle changes below thresholds that severely impact domestic activities and will additionally provide hemisphere-specific results through measuring the performance of the contralateral limb. Therefore, we expect these tests to be more sensitive than the conservative estimates used above.

With a similar reasoning, and based on region-of-interest (ROI)-wise cortical thickness data of 3T MRI of a local ALS cohort with less strict inclusion criteria, see (Schuster et al., 2014), of 130 patients, differences between cross-sectional motor cortex thicknesses of the purely single supply and the purely double supply groups can be expected to become significant at $p = 0.05$ with a power of 80% for 4 patients, consequently 8 hemispherical datasets, in each group, making a total cohort size of at least 19 patients necessary (group S mean [SD] 2.28 [0.10] mm, group D 2.43 [0.17]). We expect that the results in a more stratified cohort and with the higher resolution made possible by ultrahigh-field 7T MRI should be even more conclusive.

To detect the longitudinal stability of the supply classifications via changes in the pattern frequency using Fisher's exact test, at least $n = 6$ patients are required to detect significant changes resulting from at least one changed pattern in one hemispheres of one of the patients at $p = 0.05$.

From the power analyses we conclude that a cohort size of 20 patients matching the above-mentioned inclusion criteria should be sufficient for all planned modalities to investigate the hypotheses.

- **WP2: Imaging, pattern assessment and VDM**

  a) Image Acquisition (PhD candidate)
  For the high resolution required to image the vascular patterns of interest, the sensitivity to and likelihood of head motion-induced image artifacts (leading to effective resolution loss) increases. For sub-millimeter imaging, even in healthy young volunteers, motion-induced blurring occurs because the voxel size and amplitude of unintentional motions (i.e. breathing or muscle relaxation) are on the same order of magnitude (Herbst et al., 2014; Stucht et al., 2015). Arguably, for ALS patients it is even more challenging to remain motionless during the several tens of minutes of scanning required for high-resolution MRI. To prevent motion artifacts and the resulting decrease of effective image resolution, prospective motion correction (PMC) has been developed (Stucht et al., 2015). It was shown to be particularly effective in correcting high-resolution MRI at 7T (Lüsebrink et al., 2017). To correct motion prospectively, we use an optical tracking system to obtain real-time motion estimates and correct during scanning (Maclaren et al., 2012). To that end, the in-bore tracking system captures a marker attached to subject via an individually made mouthpiece. With this PMC system, we were able to acquire anatomical data with up to 250 µm and vascular data with up to 150 µm isotropic resolution in healthy volunteers, respectively (Lüsebrink et al., 2017; Mattern et al.,

2018), representing some of the highest resolution MR image human brain data acquired *in vivo* to date.
To illustrate the high-resolution capabilities of motion-corrected 7T MRI for this project, we acquired 700 µm MPRAGE and 250 µm ToF MRA in one healthy volunteer (see **Figure 5**).
To assess vascular function locally, CVR is estimated from a 5-minute resting state (rs) fMRI scan. The coefficient of variations of the BOLD time series correlates strongly with breath-hold CVR estimates (Jahanian et al., 2017) and, therefore, is a task-free and equipment-free CVR proxy, ideal in the context of aggravated hardware requirements at 7T and potentially non-compliant subjects.
All sequences of the 60 minutes-protocol should be well tolerated by ALS patients and enable the depiction of the motor cortex' structure and arterial vasculature at high resolution. Hence, double and single supply patterns can be identified reliably within clinically acceptable measurement time.
The patients will receive a follow-up 7T MRI after 12 months, to investigate the stability of supply patterns and to enable longitudinal structural and functional analysis of the motor cortex and further brain regions ("transfer effects", please see below).

b) Automatic and manual segmentations (clinical scientist)
The motor cortex will be localized in the structural MPRAGE sequences using established segmentation algorithms (Freesurfer), while the ACA/MCA vessel tree will be manually segmented up to the small terminal cortical branches in each individual subject, ALS patients and controls, in the co-registered ToF MRA and the MPRAGE sequences. The overlap between the motor cortex and the individual vessel watershed areas are consequently qualitatively assessed visually, leading to a corresponding supply pattern rating. Cortical thicknesses of the motor cortex, and that of other typically affected regions like the prefrontal, postcentral and temporal neocortex, as well as volumetric measurements for the basal ganglia and MTL, will be derived from processing the structural MPRAGE sequences in ALS and controls. CVR is estimated locally by computing the coefficient of variations from the rs-fMRI time series per voxel.

c) Vessel distance mapping (PhD candidate)
By applying a distance transform (Maurer et al., 2003) to each segmented feeding vessel branch, artery-specific VDMs are generated. To approximate overlapping perfusion territories from the individual VDMs, the RMS is computed. The resulting RMS artery-specific VDM returns low values for voxels close to both feeding arteries.

In order to localize regions of the motor cortex homunculus corresponding to bulbar, upper and lower limb body part areas a series of functional tests will be performed during a functional MRI (fMRI) sequence of the 7T scan in which the patient is instructed to move his tongue or mouth, certain fingers and toes (Kuehn et al., 2017; Schreiber et al., 2021). The activated areas in these fMRI scans will consequently be used to segment the motor cortex into distinct ROIs representing each body part of the motor cortex homunculus. These ROIs enable artery-specific VDM and cortical thickness to be assessed and correlated per body part.

- **WP3: Vascular resistance & resilience in ALS**

  Based upon the pattern ratings and imaging results obtained in WP2, the subsequent statistical analysis regarding the postulated hypotheses will be carried out in WP3.

  a) Test of Failure of vascular resistance in ALS (clinical scientist)
  To analyze vascular resistance against ALS pathology, differences in the frequency of double vs. single supply ACA/MCA patterns of the motor cortex between ALS and controls will be tested. The vascular patterns will be evaluated in baseline and follow-up scans to evaluate their longitudinal stability or potential alterations.

  b) Effects of supply patterns on motor function (clinical scientist)
  Direct effects of the respective motor cortex' vascular supply pattern on the local cross-sectional motor cortex thickness and distant areas ("transfer") affected in ALS will be evaluated hemisphere-wise on a group level using mixed effects linear models with hemispheric thickness and volumetric values as dependent variable, group (ALS vs. controls) and hemispheric supply pattern as fixed variables and subject identifiers as a random variable. For the ALS patients, direct vascular resilience effects of double vs. single vascular supply on current and longitudinal global motor function (ALSFRS-R total), longitudinal cortical thickness/volumetric values, CVR values, as well as mediation effects via the motor cortical thickness will be tested using similar mixed effects linear models.

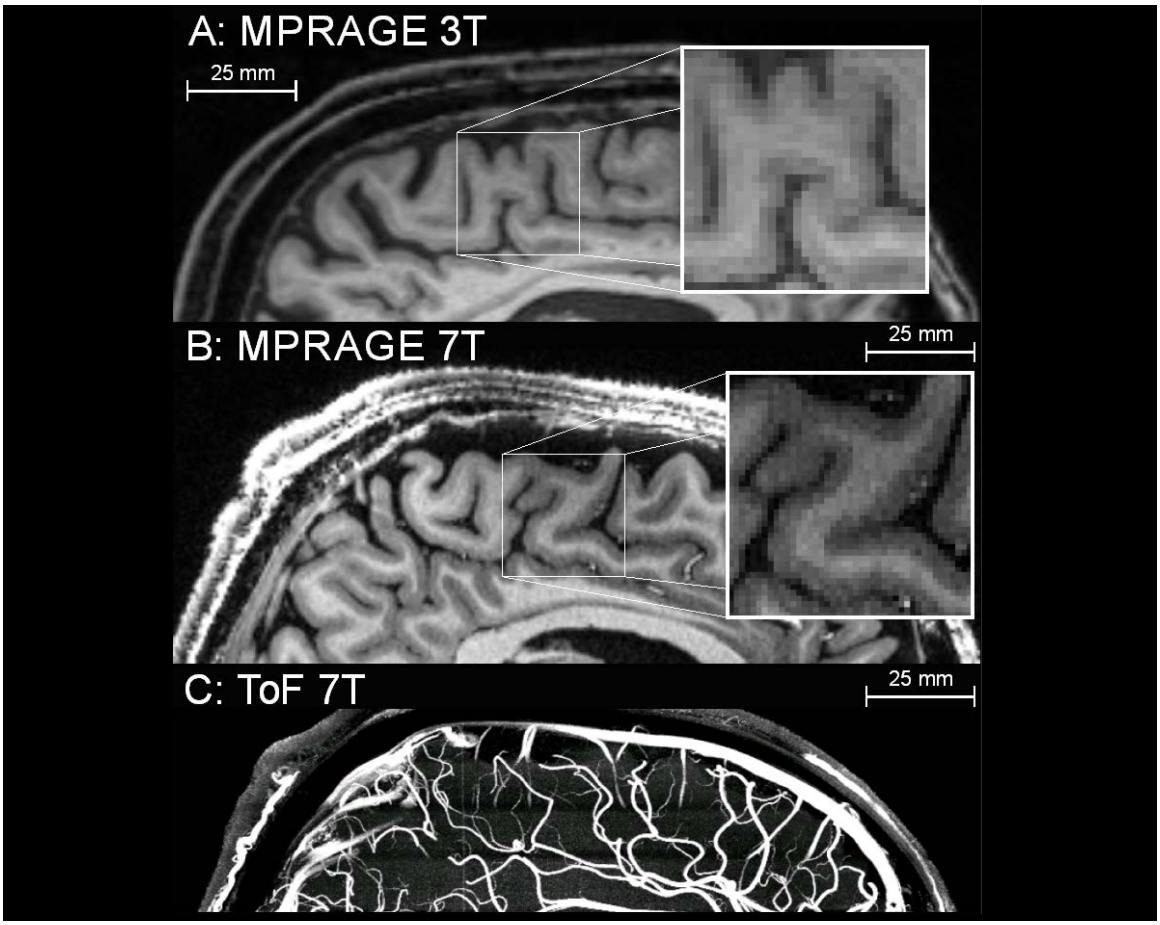

Figure 5. Comparison between structural MPRAGE sequences taken with 3T MRI (in **A**: resolution 1×1×1 mm³) and high-resolution MRI with prospective motion correction at 7T (in **B**: resolution 0.7×0.7×0.7 mm³). Note the highly improved contrast between gray and white matter, as well as the visibility of larger vessels in the MPRAGE sequences. High resolution ToF MRA (**C**) taken at 7T with prospective motion correction at a resolution of 0.25×0.25×0.25 mm³.

c) Effects of local overlap of perfusion territories on vascular resilience (PhD candidate)

Analysis of direct and mediation effects of the putative local marker for vascular supply provided by local VDM (from WP2c) on baseline and longitudinal motor cortex thickness of each body part area (bulbar, upper limb, lower limb) and current or longitudinal local motor function will be carried out on ROI-, voxel- and vertex-level using sets of mixed effects linear models. Further, potential interdependencies with CVR estimates will be investigated.

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
WP1: Recruitment and clinical testing								
5 healthy volunteers								
Recruitment ALS patients								
Scan Supervision, baseline functional test								
Follow-up functional testing								
Recruitment matched controls								
WP2: Imaging, pattern assessment, VDM								
Establishment of imaging sequences								
Baseline 7T image acquisition								
Follow-up 7T image acquisition								
Classification and Segmentation								
Vessel distance mapping								
WP3: Vascular resistance & resilience								
Vascular resistance								
Supply patterns and motor functions								
Perfusion territories and vascular resilience								
	Clinical Scientist					PhD student		

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4 Relevance of sex, gender and/or diversity

The applicants consist of female and male researchers ensuring gender equality. In the recruitment process for the PhD candidate and clinical scientist positions efforts will be undertaken to match female and male researchers.

The sex distribution of the ALS cohort affected by the classic disease phenotype studied in this project is M/F 5/4. The study cohort will be matched to approximate this ratio.

5 Supplementary information on the research context

Section 5 et seq. must not exceed 10 pages.

5.1 Ethical and/or legal aspects of the project

5.1.1 General ethical aspects

ALS is a devastating neurodegenerative disorder characterized by the progressive loss of overall muscle function leading to death by respiratory failure within 3 to 5 years after diagnosis. In ALS, risk factors, pathogenesis and disease heterogeneity have still not sufficiently been elucidated. There is no cure, and treatment merely relies on slowing of disease progression and symptom management. Based on our longstanding clinical experience, ALS patients are commonly very open-minded to take part in research studies and are highly interested in a better understanding of this fatal disease.

This study will include only cognitively normal patients/volunteers who are able to provide written informed consent. This is no interventional or clinical trial. There will be no individual benefit to the study participants. The study will be conducted in line with the national legal and ethics requirements. We already have the approval of the local ethics committee to perform 7T ultrahigh-field 7T MRI studies in ALS and aged controls (No 16/17). In order to address the new sequences carried out in the scope of this study an addendum has been approved by the local ethics committee. All participants have to read an information sheet in a language and in terms they can fully understand and they have to give their written informed consent. Participants can withdraw their consent without any reasons or consequences during the course of the study.

All data of the participants will be treated in line with the local data protection and management requirements. Pseudonymized data will be stored electronically and accessible only to people with permission for a 10-year-period.

Publication of the participants' data will not lead to a breach of agreed confidentiality and anonymity.

5.1.2 Descriptions of proposed investigations involving experiments on humans or human materials

All participants will undergo 7T ultra-high field MRI, which is considered as safe as lower field MRI (1.5T, 3T). Subjects will be carefully screened for the existence of 7T MRI contraindications, according to the recommendations of the German Ultrahigh-Field Imaging (GUFI) network, which will lead to their exclusion from the study. Side effects of ultra-high magnetic field exposure (such as vertigo or metallic taste) are transient and not harmful (Landesamtes für Verbraucherschutz Sachsen-Anhalt, 2005). Established scanning and subject preparation methods are followed. ToF MRA is a non-invasive method and there is no need for the application of contrast medium. All clinical tests conducted for research purposes are non-invasive; invasive diagnostics (e.g. electromyography) will be merely applied for diagnostic work-up as part of the clinical routine to exclude concurrent diagnoses in ALS.

5.1.3 Descriptions of proposed investigations involving experiments on animals

None.

5.1.4 Descriptions of projects involving genetic resources (or associated traditional knowledge) from a foreign country

None.

5.1.5 Descriptions of investigations involving dual use research of concern, foreign trade regulations

None.

5.2 Data handling

Data will be handled in accordance with “Leitlinie für das Forschungsdatenmanagement” of the Otto-von-Guericke University Magdeburg. All MR data acquired will be stored and processed in pseudonymized on computers of the Otto-von-Guericke University Magdeburg. Further, all unprocessed, pseudonymized MR images will be archived in the PACS of the Leibniz Institute for Neurobiology (routine back-up procedure).

5.3 Other information

Please use this section for any additional information you feel is relevant which has not been provided elsewhere.

None.

6 People/collaborations/funding

6.1 Employment status information

For each applicant, state the last name, first name, and employment status (including duration of contract and funding body, if on a fixed-term contract).

Schreiber, Stefanie, permanent, full-time

Mattern, Hendrik, fixed-term paid by Otto-von-Guericke University Magdeburg directly (contract until 15.02.2023), full-time. Extension of the position is possible.

Speck, Oliver, permanent, full-time

6.2 First-time proposal data

Only if applicable: Last name, first name of first-time applicant

Schreiber, Stefanie

6.3 Composition of the project group

List only those individuals who will work on the project but will not be paid out of the project funds. State each person's name, academic title, employment status, and type of funding.

Schreiber, Stefanie, Prof. Dr. med. habil., permanent, full-time, Senior Neurologist (Oberärztin), Department of Neurology, Otto-von-Guericke University Magdeburg

Mattern, Hendrik, Dr.-Ing., fixed-term (contract until 15.02.2023), full-time, paid by Otto-von-Guericke University Magdeburg directly

Speck, Oliver, Prof. Dr. rer. nat. habil., permanent, full-time, Director of the Department Biomedical Magnetic Resonance, Otto-von-Guericke University Magdeburg

6.4 Researchers in Germany with whom you have agreed to cooperate on this project

None.

6.5 Researchers abroad with whom you have agreed to cooperate on this project

None.

6.6 Researchers with whom you have collaborated scientifically within the past three years

This information will help avoid potential conflicts of interest.

Prof. Dr. med. Emrah Düzel (German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany)

Prof. Dr. med. Michael Sendtner (Institute of Clinical Neurobiology, University Hospital Würzburg, Germany)

Prof. Peter J. Nestor, MD, PhD (Queensland Brain Institute, University of Queensland, Australia)

Prof. Michael S. Cartwright, MD (Dept. of Neurology, Wake Forest School of Medicine, North Carolina, USA)

Prof. Dr. med. Susanne Petri (Dept. of Neurology, Hannover Medical School, Germany)

Prof. Dr. med. Johannes Prudlo (Dept. of Neurology, Rostock University Medical Center, Germany; DZNE, Rostock, Germany)

Dr. rer. nat. Esther Kühn, (Institute for Cognitive Neurology and Dementia Research, Otto-von-Guericke University Magdeburg, Germany)

Prof. Dr. Roxana O. Carare MD, PhD (Dept. of Clinical Neuroanatomy, University of Southampton, UK)

Prof. Mark Ladd, PhD (Deutsches Krebsforschungszentrum Heidelberg, Germany)

Prof. Dr. rer. medic. Harald H. Quick (Erwin L. Hahn Institute, University Duisburg-Essen)
Prof. Dr. rer. nat. Thomas Ernst (Center for Advanced Imaging Research, University Maryland, Baltimore, USA)
Prof. Dr. Maxim Zaitsev, PhD (Center for Medical Physics and Biomedical Engineering, University Vienna, Austria)
Prof. Dr. Viktor Scherer (Dept. of Energy Plant Technology, Ruhr-University Bochum, Germany)
Prof. Jonathan Polimeni, PhD (Athinoula A. Martinos Center, MGH Boston, USA)
Aswin Hoffmann, PhD (Helmholtz-Zentrum Dresden-Rossendorf, Germany)
Prof. Dr. rer. nat. Jan-Bernd Hövener (Dept. of Radiology, University Medical Center Schleswig-Holstein, Kiel, Germany)

6.7 Project-relevant cooperation with commercial enterprises

If applicable, please note the EU guidelines on state aid or contact your research institution in this regard.

None.

6.8 Project-relevant participation in commercial enterprises

Information on connections between the project and the production branch of the enterprise

None.

6.9 Scientific equipment

List larger instruments that will be available to you for the project. These may include large computer facilities if computing capacity will be needed.

The research group “Biomedical Magnetic Resonance” has direct access to a research-only 7T whole-body MR scanner (Siemens Healthcare, Erlangen, Germany) operated by the Leibniz Institute for Neurobiology (part of the Gerätezentrum "Combinatorial NeuroImaging” (CNI) listed in the RIsource catalog). Further, they have a dedicated server on-site (24 CPUs, 128 GB RAM, funded by expired DFG and BMBF grants) to process large-scale datasets. The Department of Neurology maintains a dedicated server system including PACS-, remote access SFTP- and VNC servers (32 CPUs, 128 GB RAM, 48 TB drive space).

6.10 Other submissions

List any funding proposals for this project and/or major instrumentation previously sub-mitted to a third party.

None.

7 Requested modules/funds

Explain each item for each applicant (stating last name, first name).

7.1 Basic Module

7.1.1 Funding for Staff

Clinical scientist 50% 2 years (€ 48,750 x 2)

PhD candidate 65% 2 years (€ 45,435 x 2)

The funds to employ the clinical scientist (€ 97,500) are requested by Prof. Dr. Schreiber, while the funding for the PhD candidate shall be distributed between Dr. Mattern (32%, € 44,736) and Prof. Dr. Speck (33%, € 46,134)

Sum: € 184,600

7.1.2 Direct Project Costs

7.1.2.1 Equipment up to € 10,000, Software and Consumables

To enable high performance prospective motion correction, a rigid coupling between tracking marker and the subject's skull is imperative. By attaching the marker via an individually built mouthpiece to the teeth of the upper jaw, a rigid coupling is ensured. Each mouthpiece is custom-built based on the respective subject's teeth using dental impressions. Each subject-specific, mouthpiece cost € 100, totaling € 4,500 for all 45 subject (5 subjects for piloting, 20 ALS patients, 20 healthy controls). The funds for the mouthpieces will be shared equally between Dr. Mattern (€ 2,250) and Prof. Dr. Speck (€ 2,250).

Sum: € 4,500

7.1.2.2 Travel Expenses

Clinical scientist:

33nd ALS/MND San Diego, USA, December 2022:

€ 400 Registration + € 2,000 Travel & Lodging

PhD candidate:

ISMRM 31st Annual Meeting & Exhibition, Toronto, ON, Canada, June 2023:

€ 450 Registration + € 2,000 Travel & Lodging

The funds for the travel expenses of the clinical scientist (€ 2,400) are requested by Prof. Dr. Schreiber, while the travel expenses of the PhD candidate will be equally distributed between Dr. Mattern (€ 1225) and Prof. Dr. Speck (€ 1225).

Sum: € 4,850

7.1.2.3 **Visiting Researchers** (excluding Mercator Fellows)

None.

7.1.2.4 Expenses for Laboratory Animals

None.

7.1.2.5 Other Costs

7T MRI will be performed in 45 subjects, the 20 ALS patients receiving two scans, with each subject spending 1.5 hours in the device. Due to the Corona-virus additional 30 min for cleaning disinfection are required (specified by institution-internal regulations), resulting in scanning costs of € 38,675 (€ 297.50/scan hour × 2 hours × 65 sessions).

Compensation will be provided for the 20 matched controls and the five subjects required for sequence piloting for the MRI and its preparations. Twenty ALS patients will undergo the MRI scans and will consequently carry out 5 hours for motor function and further clinical/neuropsychological testing at their baseline and final visit. Each of the patients will receive three additional follow-up visits of 4 hours (motor function and clinical testing). The baseline and follow-up scans will be compensated with € 35 per 7T scan session and the functional tests with € 10/testing hour. The incurred costs for direct subject compensation are € 6,675.

In order to select enough ALS patients fulfilling the tightly defined characteristics, patients will be recruited in Magdeburg, as well as in cooperating referral centers in Hannover and Rostock. To allow the participation of these (in some cases severely disabled) patients, travel and housing costs will have to be covered for the patient and a caregiver, if necessary.

Traveling costs are expected to reach € 20,000 (average € 200/visit), while housing costs are expected at about € 20,000 (average € 100/night, two nights for baseline examination, one for follow-ups).

Matched controls and the five subjects required for sequence piloting will be recruited from the cohort at the DZNE/Department of Neurology. In order to cover travel costs of the expectedly rural population a refund of € 0.30 / km will be provided resulting in approximately € 375 (average total 50 km travel).

The funds for patient/control compensation, housing and travel (€ 47,050) are requested by Prof. Dr. Schreiber, while the projected scanning costs will be equally distributed between Dr. Mattern (€ 19,338) and Prof. Dr. Speck (€19,338).

Sum: € 85,725

7.1.2.6 Project-related Publication Expenses

Funds of € 1,500 (€ 750/year) are requested, which will contribute to covering article processing charges to publish the findings of the project. These funds will be equally distributed between the applicants as follows: € 500 Prof. Dr. Schreiber, € 500 Dr. Mattern, € 500 Prof. Dr. Speck.

Sum: € 1,500

7.1.3 Instrumentation

7.1.3.1 Equipment exceeding € 10,000

None.

7.1.3.2 Major Instrumentation exceeding € 50,000

None.

7.2 Module Temporary Position for Principal Investigator

None.

7.3 Module Replacement Funding

None.

7.4 Module Temporary Clinician Substitute

None.

7.5 Module Mercator Fellows

None.

7.6 Module Workshop Funding

None.

7.7 Module Public Relations Funding

None.