From the Department of Neurology

Director: Prof. Dr. med. Alexander Storch

# Correlations of clinical and cognitive characteristics with neuroimaging in patients with amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS)

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The present cumulative dissertation is based on the research results from previously published articles in the journals “Journal of Neurology” and “Journal of Neurological Sciences”. In the following, the research results are presented in a shortened and summarised form, whereas the detailed results are included in:

Study I:

Lehto A, Schumacher J, Kasper E, Teipel S, Hermann A, Kurth J, Krause B and Prudlo J (2024) Cerebral glucose metabolic correlates of cognitive and behavioural impairments in amyotrophic lateral sclerosis. J Neurol 271: 5290-5300. https://doi.org/10.1007/s00415-024-12388-z

Study II:

Lehto A, Schumacher J, Kasper E, Teipel S, Hermann A and Prudlo J (2024) Loss of the ipsilateral silent period in amyotrophic lateral sclerosis is associated with reduced white matter integrity in the motor section of the corpus callosum. J Neurol Sci 466:123267. https://doi.org/10.1016/j.jns.2024.123267

Study III:

Lehto A, Schumacher J, Kurth J, Krause B, Kasper E, Teipel S and Prudlo J (2025) Inconsistent primary motor cortex glucose hypometabolism in primary lateral sclerosis. J Neurol 272, 410. https://doi.org/10.1007/s00415-025-13089-x

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# Abbreviations

| | |
|---|---|
| ADNI | Alzheimer's Disease Neuroimaging Initiative |
| ALS | Amyotrophic lateral sclerosis |
| ALSbi | ALS with behavioural impairment |
| ALScbi | ALS with cognitive and behavioural impairment |
| ALSci | ALS with cognitive impairment |
| ALSFRS-R | ALS Functional Rating Scale Revised |
| ALSni | ALS without cognitive and/or behavioural impairment |
| ANCOVA | Analysis of covariance |
| ANOVA | Analysis of variance |
| BF | Bayes factor |
| ECAS | Edinburgh Cognitive and Behavioural ALS Screen |
| FDG | 2-[$^{18}$F]Fluorodeoxyglucose |
| FSL | FMRIB Software Library |
| FTD | Frontotemporal dementia |
| HC | Healthy controls |
| iSP | Ipsilateral silent period |
| LMN | Lower motor neuron |
| MRI | Magnetic resonance imaging |
| PET | Positron emission tomography |
| PLS | Primary lateral sclerosis |
| PVE | Partial volume effect |
| PVEC | Partial volume effects correction |
| SPM | Statistical Parametric Mapping |
| TDP-43 | Transactive response DNA binding protein 43 kDa |
| TIV | Total intracranial volume |
| TMS | Transcranial magnetic stimulation |
| UMN | Upper motor neuron |

# 1. Introduction

## 1.1. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterised by a loss of motor neurons and its incidence in Europe is estimated at two to three cases per 100,000 individuals [1]. The peak incidence is estimated at 70–74 years for men and 65–69 years for women [1], the median time between symptom onset and a definitive diagnosis is 10–16 months [2] and death occurs generally within 2 to 4 years of diagnosis most commonly due to respiratory failure [3]. The characteristic combination of upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction starts in the cervical, thoracic or lumbar segment and spreads progressively to the remaining segments, most often in an anatomically contiguous manner (e.g. from lower limbs to upper limbs to the bulbar region) [4]. This leads to a progressive weakness of voluntary skeletal muscles involved in limb movement, swallowing, speaking and respiratory function. As a group, ALS patients nevertheless display considerable heterogeneity in their clinical presentation with respect to the site of disease onset, speed of symptom progression and distribution of upper and lower motor neuron signs [5, 6].

The diagnosis of ALS relies primarily on a clinical assessment. The commonly used revised El Escorial diagnostic criteria [7] provide categories of diagnostic certainty from possible through probable to definite ALS, considering the number of affected segments and the available supporting neuroimaging, laboratory and electrophysiological findings. The recent years have shown an increased interest in emerging diagnostic, categorical and prognostic biomarkers to aid in the diagnosis, identify meaningful disease subgroups, predict individual patients' prognosis and serve as outcomes in therapeutic trials [8].

One specific diagnostic biomarker of ALS, for instance, is the disruption of transcallosal inhibitory signalling, which can be measured with transcranial magnetic stimulation (TMS) [9, 10]. The coordination of voluntary movement relies on communication between the bilateral motor regions, which also involves inhibitory signalling via the corpus callosum. Deficits in transcallosal inhibition are common in ALS patients but very rare in the healthy population and have therefore been suggested to have a high clinical value as an early diagnostic marker [9–14]. In cases with low diagnostic certainty based on clinical examination, assessing transcallosal inhibition may offer valuable information [9–11]. It is unclear, however, whether the transcallosal inhibition deficit identifies an informative disease subgroup (categorical biomarker) or may be related to individual patients' prognosis (prognostic biomarker). Furthermore, previous studies have not yet demonstrated a neural correlate for this functional deficit [9, 12].

Besides the motor symptoms that are central to ALS, 30-50% of ALS patients show mild cognitive impairments and 25-70% show behavioural changes [15]. Furthermore, up to 15% of patients meet the Rascovsky criteria [16] for a behavioural variant of frontotemporal dementia (bvFTD) [15] or the Gorno-Tempini criteria [17] for primary progressive aphasia. The common way to classify cognitive and behavioural impairments in ALS is by using the revised Strong criteria [18], which yields the following categories: patients without cognitive and/or behavioural impairment (ALSni), patients with cognitive impairment (ALSci), patients with behavioural impairment (ALSbi) and patients with cognitive and behavioural impairment (ALScbi). The criteria for classifying cognitive or behavioural impairment are outlined in figure 1.

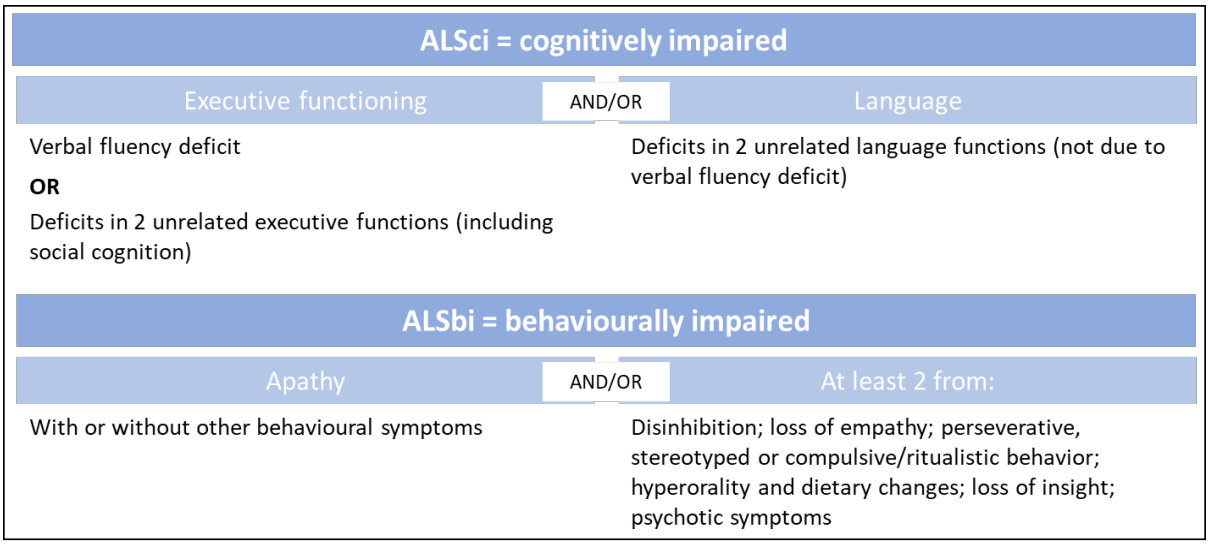


**Figure 1** The revised Strong criteria for cognitive and behavioural impairment in ALS

The cognitive domains characteristically affected in ALS include verbal fluency, executive functioning, social cognition and language [19, 20]. Behavioural changes commonly include apathy, irritability, impulsivity, lack of foresight and planning, reduced concern for hygiene and increased self-centeredness [15, 21, 22]. The accumulating evidence suggests that these cognitive and behavioural changes that do not meet the criteria for FTD can be present from the early stages of ALS, but in the majority of cases no continuous deterioration over the course of the disease takes place [23]. Nevertheless, the presence of cognitive or behavioural impairments is a predictor for a poorer prognosis with faster progression of motor symptoms and reduced survival [24, 25].

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is a neuropsychological instrument for detecting the specific profile of cognition and behaviour changes in ALS [26]. It was designed to account for the motor impairments of ALS patients, which can complicate the scoring and interpretation of other tests. The ECAS has been established in clinical settings internationally, but its acceptance in research contexts has been less uniform. As a brief screening instrument, it offers less detailed information than a time-intensive neuropsychological test battery. Therefore,

the suitability of the ECAS for research endeavours focused on cognition needs to be critically considered.

## 1.2. Primary lateral sclerosis

Primary lateral sclerosis (PLS) is characterised by a pure UMN phenotype and is considerably less prevalent than ALS, the estimations for its incidence ranging from 0.1 to 0.6 per 100,000 people per year [27, 28]. The mean age of onset is around 50 years [29] and the disease progression is conspicuously slower than ALS with an average disease duration ranging from 7.2 to 14.5 years [30]. In 90% of the cases, the symptoms begin insidiously in the lower limbs, often characterised by disequilibrium, stiffness of gait and loss of fluid movement [27]. The remaining 10% of the patients present with bulbar onset and dysarthria, which is often accompanied by pseudobulbar affect (uncontrollable crying or laughing that is out of context in the social situation) and pathological yawning [27]. Similarly to ALS patients, the most common cognitive and behavioural deficits in PLS include executive dysfunction, verbal fluency, language, apathy and impaired social cognition [31].

Numerous diagnostic criteria have been proposed over the last decades [29], illustrating the challenges of delineating PLS as its own neurological entity in the context of numerous conditions sharing its clinical phenotype (for instance UMN-dominant ALS, hereditary spastic paraplegia and progressive supranuclear palsy [27, 29, 32]). The most recent consensus diagnostic criteria [33] feature two categories: probable PLS diagnostic category for patients with UMN symptoms in at least 2 of 3 regions (lower extremity, upper extremity and bulbar) and no active LMN degeneration for 2–4 years and definite PLS diagnostic category for patients who meet these criteria for more than 4 years. Since diagnostic biomarkers of PLS are lacking, the differential diagnosis still necessitates a broad range of assessments (e.g. magnetic resonance imaging, denervation potentials on electromyography, motor evoked potentials and cortical excitability assessment with TMS) [32, 34].

## 1.3. Neuroimaging in ALS

Neuroimaging has clinical value for excluding possible alternative diagnoses, but neuroimaging research also has the special potential to delineate the disease-specific neurodegeneration patterns and visualise the heterogeneity in patients' clinical presentation.

The literature on structural and functional brain changes in ALS compared to healthy aging or different neurological conditions is extensive [15, 35–37]. Based on magnetic resonance imaging (MRI) data, both the regional grey matter metrics and the white matter connectome have been found altered in ALS patients and those changes correlate with disease progression and severity [38–40]. The characteristic findings include damage to the primary motor cortex, the corpus

callosum (in particular, the middle and posterior parts) and the corticospinal tract, but regional alterations have been described in all lobes with the exception of the occipital lobe as well as in subcortical regions such as the basal ganglia, thalamus and midbrain. Various neurodegenerative changes beyond motor regions have been found to correspond well with patients' non-motor symptoms [15, 35, 41].

Some neuroimaging studies have focused specifically on the neural correlates of cognitive deficits. ALS patients with cognitive impairment show more pronounced anatomical alterations than patients without cognitive impairment, characterised by reduced grey matter volume and cortical thickness in the frontal and temporal lobes, insular and cingulate cortices, cerebellum and basal ganglia in addition to greater white matter changes in the corticospinal tract, corpus callosum, cingulum, inferior longitudinal tract, inferior fronto-occipital tract and uncinate fasciculi [15]. However, the criteria for defining cognitive deficits differed from study to study and the number of investigations using the current standard neuropsychological instrument ECAS is limited.

Besides MRI, radiotracer imaging has been informative in illustrating the neurodegeneration patterns of ALS patients. For instance, positron emission tomography (PET) imaging with the radiotracer 2-[$^{18}$F]Fluorodeoxyglucose (FDG) is commonly used to investigate differences in regional glucose uptake by neurons and astrocytes [42, 43]. The regions with the starkest structural changes in MR studies are also consistently reported as hypometabolic in ALS: the motor cortex and prefrontal cortex [44–46]. Regions reported as hypermetabolic include the cerebellum, occipital cortex, midbrain and medial temporal cortex [44–48]. The presence of cognitive and behavioural impairments correlates strongly with the spread and severity of frontal hypometabolic changes [49]. However, investigations about associations between glucose metabolism and specific cognitive domains relevant in ALS such as executive functioning, verbal fluency and social cognition are still scarce [50].

Each imaging method has its pitfalls and one of the challenges of PET is the low spatial resolution that leads to partial volume effects (PVEs). PVEs describe a phenomenon whereby the signal measured in a given brain region reflects a combination of the true regional signal and the signal from neighbouring tissues or cerebrospinal fluid, leading to blurring [51]. For a radiotracer like FDG, the PVEs lead to an underestimation of the true grey matter signal at the tissue boundaries due to the lower signal from the white matter or cerebrospinal fluid. The extent of PVEs is dependent on various factors including the scanner resolution, the size of the measured region and the presence of atrophy [51]. There is no agreement in the literature whether carrying out a PVE correction (PVEC) is necessary in ALS and it has not been tested whether metabolic measurements in ALS patients are affected by PVEs.

### 1.4. Neuroimaging in PLS

Structural neuroimaging studies in PLS samples have described a signature neurodegeneration pattern that portrays the characteristic UMN degeneration: grey matter atrophy in the primary motor cortex [52–55] combined with white matter damage in the corpus callosum [52, 53, 55, 56] and the corticospinal tracts [52, 55, 57]. Besides these changes established in the literature, a recent longitudinal investigation further described grey matter volumetric decreases in supplementary and pre-motor cortex in addition to a progressive decrease in cortico-medullary and inter-hemispheric structural connectivity [55]. Further alterations have been reported in the brainstem, subcortical grey matter and cerebellum [58]. Overall, the cerebral imaging signature of PLS is comparable to ALS [58].

Therefore, despite the establishment of PLS-characteristic MRI changes, further sensitive diagnostic tools would be valuable. Case reports have described localised primary motor cortex glucose hypometabolism in PLS patients [59–62], which would support the use of FDG-PET as a supporting assessment in the differential diagnosis. The reliability of this promising finding in a consecutive sample of PLS patients, however, has not yet been inspected.

## 2. Research aims

The overarching aim of this doctoral thesis was to address currently relevant open questions pertaining to the structural and glucose metabolic alterations in ALS and PLS and their clinical manifestations, whether motoric, cognitive or neuropsychiatric in nature.

### 2.1. Neural correlates of cognitive and behavioural deficits in ALS (study 1)

The main aim of the first study was to examine differences in glucose metabolism between ALS patients with and without cognitive or behavioural impairment. Differently to a previous publication that used a time-intensive comprehensive neuropsychological test battery [49], the ECAS cognitive screening instrument that is the current standard in clinical practice was applied. Additionally, I intended to investigate associations between patients' glucose metabolism and their performance on various cognitive domains measured by ECAS. Since the influence of PVEs on FDG analyses in ALS samples had not been assessed, I also examined the impact of PVE correction on FDG-PET findings.

Furthermore, the grey matter volume differences between ALS patients with and without cognitive or behavioural impairment in addition to the correlations between patients' regional grey matter volume and their performance on various cognitive domains measured by ECAS were of interest.

## 2.2. Transcranial inhibition deficit in ALS and its neural correlate (study 2)

The main objective of the second study was to evaluate the value of a transcallosal inhibition deficit as a categorical or prognostic biomarker in ALS by comparing the demographic, clinical and neuropsychological profiles of ALS patients with intact and deficient transcallosal inhibition. Moreover, I aimed to determine the neural substrate for this functional impairment by using a region-specific approach in contrast to previous reports.

## 2.3. Glucose metabolic changes as a diagnostic biomarker in PLS (study 3)

The aim of the third study was to examine the reliability of FDG-PET imaging as a diagnostic tool in PLS by assessing the scans for primary motor cortex hypometabolism in a consecutive sample of PLS patients. Additionally, the longitudinal changes in glucose metabolism over the course of the disease were analysed.

# 3. Methods

## 3.1. Sample and assessment procedures

### *3.1.1. Sample and clinical assessment*

The three studies presented in this thesis analysed data from three independent prospective cohorts. All patient data were collected through the research institution German Center for Neurodegenerative Diseases at the Rostock University Medical Center. Prospective data from 05/2017 to 09/2024 were used in study 1 (N = 67), study 2 (N = 89) and study 3 (N = 9). Patient data recruited from 03/2011 to 03/2013 were used in study 2 (N = 38). Lastly, data from healthy controls (HCs) were acquired from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database for study 3 (N = 70).

Exclusion criteria for all patients and HCs within the study cohorts (studies 1-3) included acute or past traumatic brain injury, previous neurovascular damage, epilepsy or a history of severe psychiatric illness. As additional requirements, the HCs from the ADNI database had a Mini Mental State Examination [63] score ≥ 28, were cognitively normal after a 4-year clinical follow-up and did not show amyloid PET positivity.

All patients were classified according to the revised El Escorial criteria [7] for ALS (studies 1 and 2) or the consensus diagnostic criteria [33] for PLS (study 3). Additionally, the comorbid diagnosis of frontotemporal dementia or primary progressive aphasia was determined based on the Rascovsky criteria [16] or the Gorno-Tempini criteria [17], respectively (studies 1 and 2). All patients received a clinical and neurological examination, which included the ALS Functional Rating Scale Revised (ALSFRS-R) [64].

### *3.1.2. Neuropsychological assessment*

Neuropsychological data from various tests were analysed for studies 1 and 2 (Table 1).

| **Test instrument** | **Cognitive domain/ Behaviour** | **Inclusion in thesis studies** |
|---|---|---|
| Montreal Cognitive Assessment [65] | Short cognitive screening test including visuospatial, executive, language, memory, attention and orientation measurement of approximately 10 minutes | Study 2 |
| Edinburgh cognitive and behavioural ALS screen [26, 66] | Expanded cognitive screening test including language, verbal fluency, executive, memory and visuospatial measurement | Study 1 and study 2 |
| Faux-Pas test [67] | Social cognition: theory of mind | Study 1 |
| Facial Recognition Task [68] | Social cognition: identifying the emotions of others based on facial expressions | Study 1 |
| Informant version of the Frontal System Behaviour Scale [69] | Behavioural changes in apathy, disinhibition and executive dysfunction | Study 1 and study 2 |

**Table 1** The neuropsychological tests applied in the studies.

### *3.1.3. TMS assessment*

In study 2, a TMS measurement was undertaken to estimate the transcallosal inhibition. After determining the point of optimal excitability in the primary motor cortex, motor evoked potentials (MEP) were recorded from the abductor pollicis brevis or the first dorsal interosseous muscle of each hand to determine the resting motor threshold. Ipsilateral silent period (iSP) was recorded from the muscle by stimulating transcranially at 1.5 times the resting motor threshold while patients maintained a maximum tonic activation of the ipsilateral hand muscle and relaxed the contralateral hand muscle. After offline rectification, the presence of iSP was evaluated and the latency of the iSP, if present, was averaged across 10 trials per hemisphere.

## 3.2. Imaging data acquisition and pre-processing

Data from different types of imaging modalities were analysed with various methodological approaches to answer the research questions of this thesis.

### *3.2.1. MRI*

$T_1$-weighted MR images (studies 1 and 2) and diffusion-weighted images (study 2) were acquired on two scanners at the Rostock University Medical Centre (a 3 Tesla Siemens Skyra Fit scanner and a Siemens Vida scanner). For all $T_1$-weighted images, magnetization prepared rapid gradient echo sequence with isotropic voxel size of 1 $mm^3$ was used. The diffusion-weighted sequence had an isotropic resolution of 2 $mm^3$ and contained 5 b0 scans per 2 b-values (700 and 1000 $s/mm^2$) and 30 diffusion directions per b-value (700 und 1000 $s/mm^2$).

The $T_1$-weighted images were pre-processed in Statistical Parametric Mapping (SPM) 12 using Matlab (studies 1 and 2). The scans were skull-stripped, segmented and spatially normalized using the cat12 toolbox [70]. Subsequently, statistical analysis was undertaken in a voxel-based manner (study 1).

The diffusion-weighted data were processed with the FMRIB Software Library (FSL). For all scans, pre-processing included brain-tissue extraction and correction for eddy currents and motion. A bi-tensor model was fitted with the Diffusion Imaging in Python algorithm [71]. The free-water-corrected white matter metrics maps were estimated. Furthermore, tractography was used to isolate the transcallosal connections between the primary motor cortices and the free-water-corrected white matter metrics for this white matter tract were extracted.

### *3.2.2. FDG*

The PET images were acquired on a Gemini TF 16 scanner (Philips Healthcare) 30 min after the injection of FDG and static FDG-PET images were reconstructed (studies 1 and 3). Additionally, FDG-PET data of HCs were obtained in pre-processed form from the ADNI database (study 3). The ADNI images were acquired using various scanners, but with platform-specific protocols and standardised pre-processing. To achieve the same spatial resolution (6 mm) for the Rostock data that was available for the ADNI data, a Butterworth function was applied to the Rostock scans (only study 3). The further preprocessing steps for Rostock FDG-PET data included skull-stripping, intensity normalisation and spatial normalisation (studies 1 and 3).

In study 1, the FDG-PET scans were pre-processed both with and without PVEC according to the Müller-Gärtner method [72] to enable a comparison of results. Subsequently, statistical analysis was undertaken in either voxel-based manner (study 1) or the regional FDG signal uptake was extracted using the Human Motor Area Template [73] and the Brainnetome Atlas [74] (study 3).

## 3.3. Statistical analyses

Statistical analyses were carried out using several software applications: Statistical Product and Service Solutions, Jeffreys's Amazing Statistics Program, Matlab, SPM12 and R.

### *3.3.1. Study 1*

Patients were categorised into groups based on Strong criteria [18] (ALS patients with and without cognitive or behavioural impairment). The study sample was examined on demographic and clinical data and the different groups were compared using chi-squared tests and Analysis of Variance (ANOVA). The groups' scores on cognitive tests were compared with Kruskal–Wallis tests and Mann-Whitney U tests.

Group differences in imaging data (FDG-PET scans and $T_1$-weighted scans) were analysed in a voxel-based manner with one-way ANOVAs and subsequent T-contrasts. Covariates included age and sex for all analyses and the total intracranial volume (TIV) was additionally included in the analysis of $T_1$-weighted images. Further voxel-based regression analyses were executed per cognitive domain and subtest scores that differed between the groups in the preceding analysis of cognitive performance. Age, sex and years of formal education were included as covariates and TIV was additionally included in the analysis of $T_1$-weighted data.

In parallel, we replicated these analyses using a different approach of partial least squares regression with an application in Matlab [75]. This method makes use of covariance between voxels and yields latent variables that each identify a pattern of brain regions that covary with some external measure (e.g. a cognitive score or a grouping variable) [76]. The significance of each latent variable and the reliability of voxel saliences on each latent variable was determined.

### *3.3.2. Study 2*

Bayes factor (BF) hypothesis testing [77] was used for the statistical analyses in study 2 because in contrast to classical frequentist inference it quantifies both the evidence that supports and the evidence that opposes an effect of interest in the data. Therefore, the BF calculated from the data can either support the presence of an effect ($BF_{10} \geq 3$), the absence of an effect ($BF_{10} < 0.33$), or offer inconclusive evidence ($BF_{10}$ between 0.3 and 3).

The patients were categorised based on whether they showed iSP response or its loss. These groups were compared on numerous demographic, clinical and neuropsychological variables using Bayesian chi-squared tests and Bayesian t-tests. The differences in disease progression rate were examined. Besides the BFs, also the 95% credible interval of the posterior distribution of the parameter values for each variable was determined.

Group differences in the diffusivity metrics within the transcallosal tract extracted with tractography were tested in a voxel-wise manner with t-tests as well as in a region of interest manner with Bayesian univariate analysis of covariance (ANCOVA). For both types of analyses, the covariates were age, sex and the whole-brain white matter diffusivity metric to control for non-specific individual differences.

### *3.3.3. Study 3*

The regional FDG signal uptake values of the PLS patients at all available time points were converted into w-scores based on the values of the HCs. W-score denotes a z-score that has been adjusted for covariates: age and sex in this analysis. Therefore, the w-scores follow the z-distribution and are directly translatable to p-values. W-scores close to 0 represent values similar

to the average of the HCs whereas values < -1.65 indicate hypometabolism (signifying one-tailed p-value below 0.05).

# 4. Results

## 4.1. Glucose metabolic changes related to the cognitive and behavioural deficits in ALS

The ALSci patients had lower scores than ALSni on the cognitive domains of language, executive functions, verbal fluency and memory, whereas ALScbi patients had lower scores than ALSni on executive functions, verbal fluency and memory. No differences in cognitive scores were observed between ALSci and ALScbi patients.

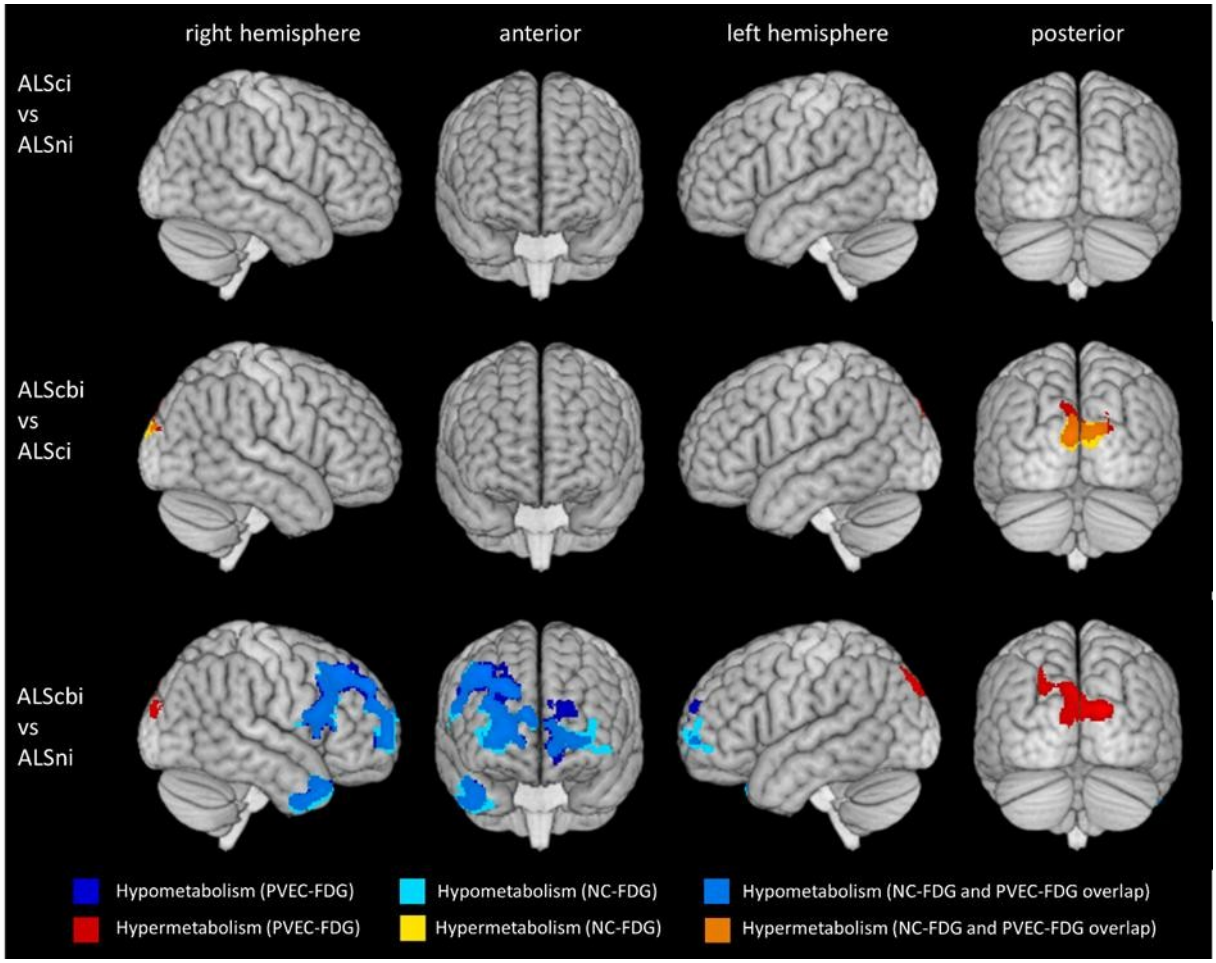


**Figure 2** Clusters of relative hypo- and hypermetabolism per group comparison.
The FDG-PET signal is relative to the average FDG-tracer uptake in GM. Height threshold is $p<0.001$ and all data are $p<0.05$ FWE-corrected at cluster level. ALSni, not impaired; ALSci, cognitively impaired; ALScbi, cognitively and behaviourally impaired; PVEC-FDG, PVE-corrected FDG-PET data; NC-FDG, non-corrected FDG-PET data.

The results of SPM analyses of FDG-PET data are illustrated in Figure 2. The analyses with and without PVEC led to similar results. No differences in FDG-PET signal were detected between

ALSni and ALSci patients in SPM or partial least squares analyses. ALScbi patients showed hypometabolic clusters in the fronto-temporal regions in addition to hypermetabolism in the occipital region when compared to ALSni patients. The partial least squares analyses corroborated these results. Compared to ALSci patients, ALScbi group showed hypermetabolism in the cuneus and occipital region and this difference was on the threshold of significance in the partial least squares analyses.

Specific clusters of hypometabolism were related to lower scores on various cognitive domains in the SPM analyses (Figure 3), whereas corresponding partial least squares analyses indicated a lack of associations. Executive functioning tasks correlated with metabolism in small regions of the frontal, lingual, cerebellar, precentral and temporal cortex. Verbal fluency scores correlated positively with metabolism in the frontal, precentral and inferior orbital regions, but negatively with metabolism in the cerebellum. Memory performance was positively associated with metabolism in the precuneus, fusiform and temporal regions. Faux Pas Test scores correlated with metabolism in the precentral and frontal areas.

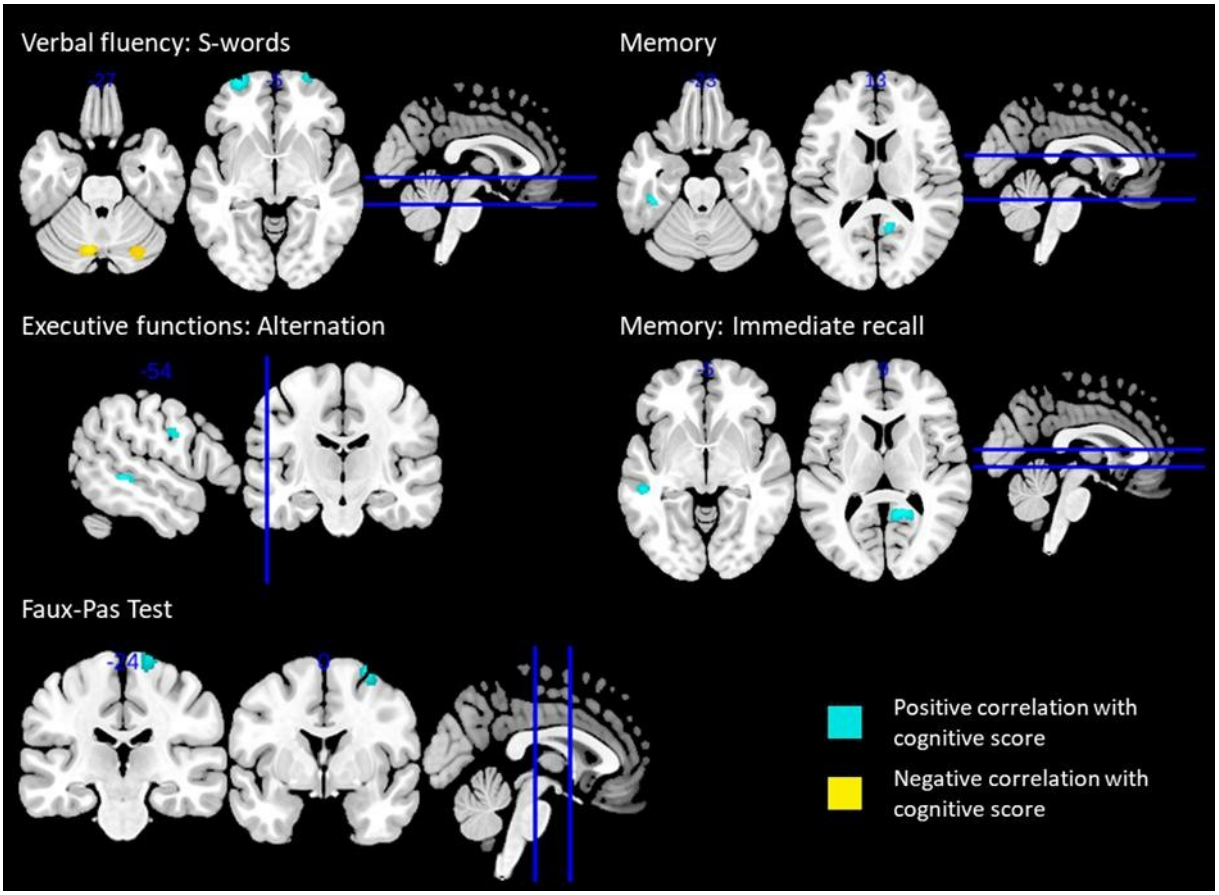


**Figure 3** Correlations of glucose metabolism and cognitive scores. The FDG-PET signal is relative to the average FDG-tracer uptake in grey matter. Height threshold is $p<0.001$ and minimum cluster size is 100 voxels.

## 4.2. Structural changes related to the cognitive and behavioural deficits in ALS

No differences in grey matter volume were detected between ALSni and ALSci patients in the SPM analysis. ALScbi patients had lower volume in the middle frontal and inferior temporal gyrus compared to ALSni. Compared to ALSci patients, the ALScbi group had lower grey matter volume in the inferior temporal gyrus.

Patients' scores on various cognitive domains were related to regional changes in grey matter volume. Higher scores for executive functioning tasks were associated with higher volume in the cerebellum. Memory performance was related to the volume of the cuneus, precuneus and hippocampus.

## 4.3. Transcranial inhibition deficit in ALS and its anatomic correlate

69 patients (54%) in our sample of 127 had a loss of iSP indicating a transcranial inhibition deficit. Six patients showed a loss of iSP only on one side. There were no differences between the groups in age, sex, phenotypic composition, years of education, the distribution of Strong classification or genetic composition. The groups did not differ in terms of any cognitive or behavioural metrics. Furthermore, there were no differences in the rate of disease progression between the groups.

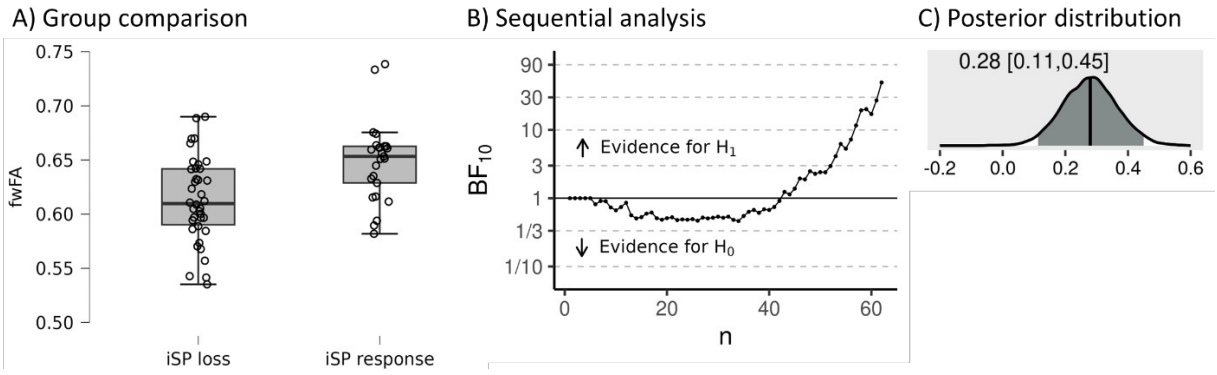


**Figure 4** Comparison of diffusivity metrics within the WM tract connecting primary motor cortices in patients with and without a loss of iSP
A) Box plots of free-water-corrected fractional anisotropy values. B) Sequential analysis demonstrating the internal coherence of Bayesian ANCOVA. C) The posterior distribution of standardised parameter estimates for the effect of group by Bayesian ANCOVA with the median and the 95% credible interval.

The diffusion-weighted data showed that ALS patients with iSP loss had lower free-water-corrected fractional anisotropy values within the tract connecting primary motor cortices (Figure 4A). The visual representation of the change in BF with every sequential inclusion of a patient showed that the presence of a group difference reached a level of strong support ($BF_{10} > 10$) with the inclusion of 58 cases and the inclusion of all cases (N=63) resulted in very strong evidence for the presence of group differences (Figure 4B). The posterior distribution for the effect of group

indicated a small effect size (Figure 4C). Voxel-level analyses of the diffusivity metrics within the tract did not result in any clusters of group differences.

## 4.4. Glucose metabolic changes as a diagnostic biomarker in PLS

The nine PLS patients included in the sample varied in their age and disease duration at baseline. At the baseline assessment, six patients already had a symptomatic involvement of all three regions (bulbar, upper limb and lower limb). Two patients completed two follow-up assessments, and three further patients completed one follow-up assessment. Two patients had an asymmetrical presentation of PLS symptoms.

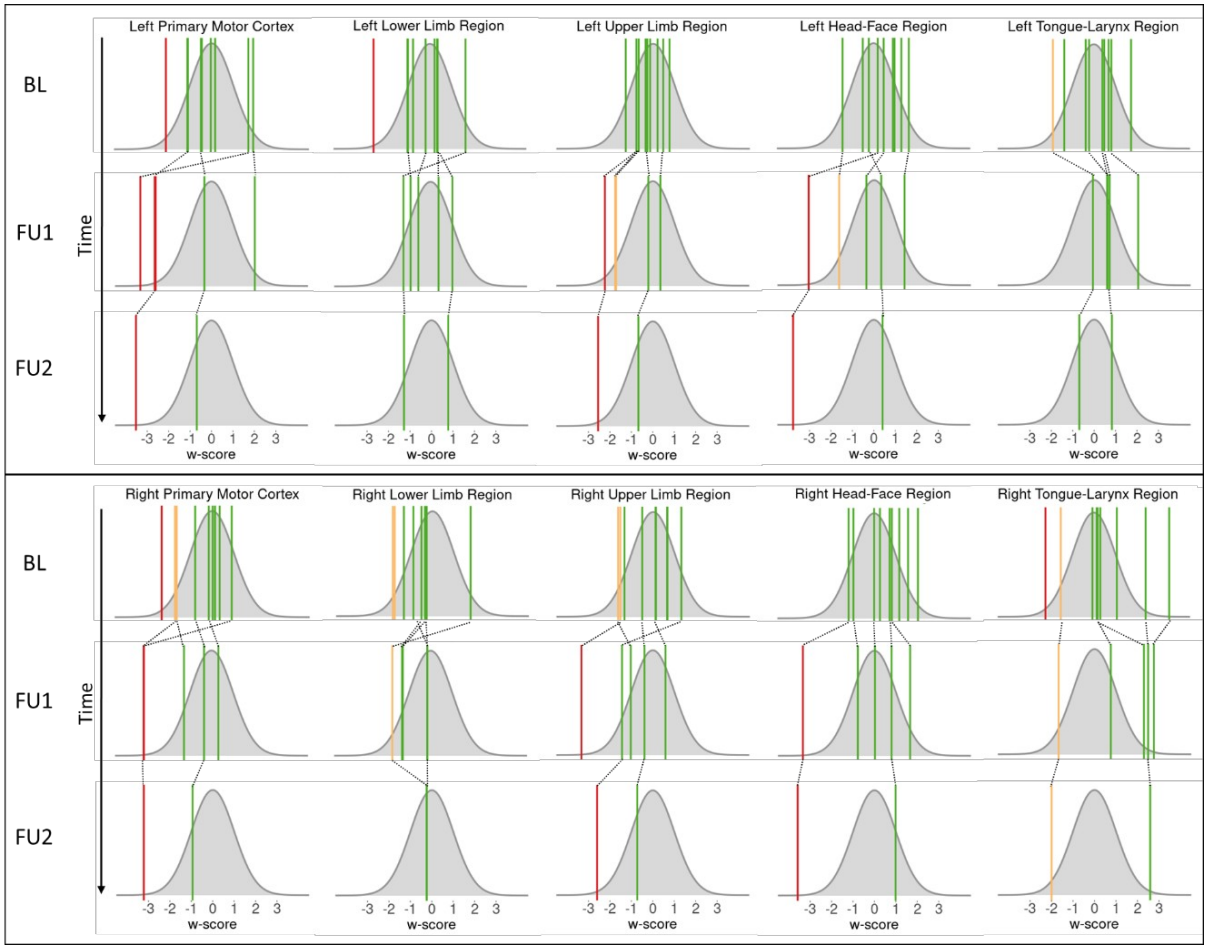


**Figure 5** Regional w-scores of PLS patients for available time points
The w-scores of PLS patients are visualised with coloured lines over a normal distribution. W-scores > -1.65 are shown in green (one-sided p-value > 0.05), w-scores between -1.65 and -1.96 are shown in yellow (one-sided p-values between 0.05 and 0.025), and w-scores < -1.96 are shown in red (one-sided p-value < 0.025). Right and left refer to the assessed hemisphere. BL, baseline; FU, follow-up.

The patients' regional w-scores were projected onto standard normal distributions in Figure 5. Despite the considerable symptomatic burden, only four of the nine patients showed regional hypometabolism in the primary motor cortex: two patients from baseline onwards and two patients only from the first follow-up onwards. Three of those four patients also showed glucose

hypometabolism in motor regions beyond the primary motor cortex. Some patients displayed a decrease in the glucose metabolism of motor regions over time whereas others maintained their levels despite the progression in symptom severity. No distinct patterns emerged between primary motor cortex glucose metabolism and clinical variables such as age, disease duration, or the spread of symptoms in the sample of PLS patients.

# 5. Discussion

## 5.1. Glucose metabolic changes related to the cognitive and behavioural deficits

My first objective was to examine the glucose metabolic changes related to the cognitive and behavioural symptoms in ALS patients. In group comparisons, I validated previous findings, which illustrated that ALScbi patients with concurrent cognitive and behavioural impairment showed widespread changes in glucose metabolism compared to ALSni and ALSci patients [49].

It seems notable that the current results seem to suggest that alterations in glucose metabolism in ALS patients may be more closely related to the presence of behavioural changes than cognitive impairments. Although the cognitive tests for the ALScbi and ALSci groups showed no differences, the ALScbi vs. ALSci comparison yielded a similar distribution of hypo and hypermetabolic clusters as the ALScbi vs. ALSni comparison. Most of the assessed cognitive domains of the ALSci vs. ALSni groups showed differences but the FDG scan comparisons did not reveal similarly located hypo or hypermetabolic clusters. This pattern of findings suggests that the glucose metabolism changes observable in ALS patients may be more indicative of behavioural impairments than specific cognitive deficits. The limited influence of cognitive impairments on glucose metabolism may be partially explained by the heterogeneous composition of this patient group as defined by Strong [18], since this category includes patients with mild deficits in either verbal fluency, executive functioning, or language – and those impairments may have different structural substrates.

To mitigate the problem of heterogeneity within the ALSci group, I also examined the glucose metabolic correlates of individual cognitive domains in the group of ALS patients as a whole. The limited associations between glucose metabolism and cognitive performance as indicated by the lack of ALSni to ALSci group differences were further underlined by the varying results from voxel-based SPM analyses and the partial least squares approach. Whereas exploratory SPM analyses identified some small clusters where glucose metabolism was related to patients' performance on specific cognitive domains, the corresponding partial least squares analyses utilising an alternative method showed a lack of associations. Therefore, changes in glucose metabolism do not seem to be strongly related to cognitive functioning in ALS patients.

As a complementary analysis, I was interested in the influence of carrying out a PVEC for the glucose metabolism results in our ALS sample. The current literature is inconsistent in this respect with some studies applying PVEC and others not. The direct comparison of findings from analyses with and without PVEC presented in this paper showed that the identified clusters overlapped to a large degree and thus indicate that the influence of PVEs on glucose metabolism estimates may be negligible.

## 5.2. Structural changes related to the cognitive and behavioural deficits

My second objective was to inspect changes in grey matter volume associated with the cognitive and behavioural symptoms in ALS patients. I replicated the common finding that ALS patients with concurrent cognitive and behavioural deficits show more anatomic alterations compared to patients without cognitive or behavioural impairments, but the extent of these differences was considerably more limited than in the FDG-PET analyses. Furthermore, correlational analyses yielded small clusters where the grey matter volume was weakly related to specific cognitive scores in our sample of ALS patients. A previous study that also employed the ECAS test found comparable results of small clusters at low significance thresholds [41]. The findings of these two studies together further strengthen the sentiment that regional grey matter volume is not strongly related to cognitive functioning in ALS patients.

## 5.3. Transcranial inhibition deficit in ALS

The third objective of this thesis was to evaluate the potential of the transcallosal inhibition deficit as a classifying biomarker in ALS in addition to identifying its structural neural correlates. First, I identified a transcallosal inhibition deficit in 54% of the ALS patients in our sample, which is comparable to previous reports [9, 12]. Second, I found no indication that this impairment would be related to meaningful demographic, clinical or neuropsychiatric phenotypic characteristics of the patients or the speed of their disease progression. This is congruent with former studies with smaller samples [9, 14, 78]. Third, thanks to a larger sample size and a hypothesis-directed region-specific analysis in contrast to a whole-brain approach [9, 12], I was able to determine a small decrease in white matter integrity within the transcallosal tract in patients with the deficit. The small effect size for this group difference despite the prevalence of the transcallosal inhibition disruption in ALS seems to indicate that even slight structural changes, which may not always be visible on standard imaging analyses, may suffice to disrupt the normal transcallosal inhibition response. Although the TMS assessment of transcallosal inhibition deficits remains a valuable diagnostic tool in ALS, the current findings suggest that it does not serve as a categorical or prognostic biomarker.

## 5.4. Glucose metabolic changes in PLS

As the fourth objective, I examined the reliability of primary motor cortex hypometabolism as a diagnostic biomarker in PLS. Despite the previous positive case reports [59–61], I detected primary motor cortex hypometabolism in only four of the nine patients, which highlights the heterogeneity within PLS. These results are counterintuitive due to the clinically conspicuous involvement of upper motor neurons in those patients, but the inter-patient variability in the current findings may have several possible explanations. First, FDG-PET may not be sensitive enough to identify limited neurodegeneration in the first stages of the disease in each PLS patient. Second, the pathologies leading to the clinical PLS phenotype in our sample may differ. Although transactive response DNA binding protein 43 kDa (TDP-43) pathology is the most common cause of PLS, the pathological variability that may lead to this phenotype (e.g. tauopathy) is well described [79–81] and the difficulties of differential diagnosis are a current topic of interest [81]. Since autopsy data were not available for our sample, I could not test any possible relationships between underlying pathology and glucose metabolism. Third, increased glial activation co-localised with grey matter atrophy [82, 83] is reflected in a higher glucose uptake [84, 85], which might mask the hypometabolism due to neuronal death in some patients. Although the diagnosis of PLS remains challenging and FDG-PET has been proposed as a potentially sensitive diagnostic tool, its utility appears limited, particularly in early disease stages when a sensitive and reliable biomarker would be most beneficial.

## 5.5. Limitations

Although the spatial resolution available in the studies presented was sufficient to identify meaningful differences, the relatively low spatial resolution of FDG-PET imaging hinders the detection of focal metabolic changes contained to small regions. Moreover, though each analysis could provide an answer to a specific research question and yield sound conclusions, our sample sizes varied greatly, and, of course, larger sample sizes always provide higher statistical power. Despite its advantages of speed of administration and suitability in ALS, the ECAS test does not offer as much detail as an extensive neuropsychological battery, which may be preferable when focusing on specific cognitive functions.

## 5.6. Conclusions and future directions

The studies included in this thesis clinical, neuropsychological, imaging and neurophysiological data from well-characterised ALS and PLS patients. The research objectives could be addressed with appropriate analyses of this valuable multimodal dataset and important insights were obtained. Key findings were as follows: the fronto-temporal glucose hypometabolism in ALS patients seems to correspond more to behavioural than cognitive impairments. Cognitive functioning in ALS patients in different domains as assessed with the ECAS instrument is not

strongly related to regional glucose metabolism or grey matter volume changes. A transcallosal inhibition deficit is associated with a reduced integrity of white matter connections between the motor cortices; however, this deficit neither corresponds with a specific phenotype of ALS nor with the rate of disease progression. Therefore, a transcallosal inhibition deficit does not serve as a categorical or prognostic biomarker in ALS. Moreover, FDG-PET does not reliably identify primary motor cortex glucose hypometabolism in PLS and has thus only limited value in a differential diagnosis. In conclusion, the current studies highlighted multiple aspects of heterogeneity among patients with ALS and PLS; the results also enhance understanding of the neuroimaging correlates of these phenotypic characteristics.

Based on the findings of this thesis, there are several interesting directions for future research. Motivated by the detected associations between glucose hypometabolism and behavioural impairment, a detailed longitudinal assessment of different neuropsychiatric changes and their neural correlates in ALS patients would be valuable. Furthermore, the apparent clinical heterogeneity within ALS and PLS patients and the increasing availability of machine learning approaches suggests that applying data-driven analysis methods to find meaningful patient profiles could result in more reliable prognostic estimates and eventual future treatments.

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# 7. Publications included in this thesis

## 7.1. Cerebral glucose metabolic correlates of cognitive and behavioural impairments in amyotrophic lateral sclerosis

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ORIGINAL COMMUNICATION

# Cerebral glucose metabolic correlates of cognitive and behavioural impairments in amyotrophic lateral sclerosis

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## Abstract

**Objective** Half of ALS patients are cognitively and/or behaviourally impaired. As cognition/behaviour and cerebral glucose metabolism can be correlated by means of $^{18}$F-Fluorodeoxyglucose positron emission tomography (FDG-PET), we aimed to utilise FDG-PET, first, to replicate group-level differences in glucose metabolism between non-demented ALS patients separated into non-impaired (ALSni), cognitively impaired (ALSci), behaviourally impaired (ALSbi), and cognitively and behaviourally impaired (ALScbi) groups; second, to investigate glucose metabolism and performance in various cognitive domains; and third, to examine the impact of partial volume effects correction (PVEC) of the FDG-PET data on the results.

**Methods** We analysed neuropsychological, clinical, and imaging data from 67 ALS patients (30 ALSni, 21 ALSci, 5 ALSbi, and 11 ALScbi). Cognition was assessed with the Edinburgh Cognitive and Behavioural ALS Screen, and two social cognition tests. FDG-PET and structural MRI scans were acquired for each patient. Voxel-based statistical analyses were undertaken on grey matter volume (GMV) and non-corrected vs. PVE-corrected FDG-PET scans.

**Results** ALSci and ALScbi had lower cognitive scores than ALSni. In contrast to both ALSni and ALSci, ALScbi showed widespread hypometabolism in the superior- and middle-frontal gyri in addition to the right temporal pole. Correlations were observed between the GMV, the FDG-PET signal, and various cognitive scores. The FDG-PET results were largely unaffected by PVEC.

**Interpretation** Our study identified widespread differences in hypometabolism in the ALScbi-ni but not in the ALSci-ni group comparison, raising the possibility that cerebral metabolism may be more closely related to the presence of behavioural changes than to mild cognitive deficits.

**Keywords** Amyotrophic lateral sclerosis · Cerebral glucose metabolism · FDG-PET · Cognition · Grey matter volume

## Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative motor neuron disorder. Besides rapidly advancing motor impairment, up to 50% of people living with ALS are cognitively impaired, up to 70% show behavioural changes, and up to 15% meet the criteria for a frontotemporal dementia [1]. The cognitive domains characteristically affected in ALS include verbal fluency, executive functioning, social cognition, and language [2, 3]. Behavioural changes include apathy, irritability, and increased self-centeredness [1, 4, 5].

Positron emission tomography (PET) imaging with the radiotracer 2-[$^{18}$F]Fluorodeoxyglucose (FDG) provides an estimation of glucose uptake by neurons and astrocytes [6, 7], enabling the investigation of differences in

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regional metabolism. The typical pattern of glucose hypo- and hypermetabolism in ALS has been well documented, with the most consistently reported hypometabolic regions being the motor cortex and prefrontal cortex [8–10]. In terms of hypermetabolism, previous studies have reported alterations in the cerebellum, occipital cortex, midbrain, and medial temporal cortex [8–12].

Associations have been reported between cognitive status and metabolic changes in ALS patients: more extensive and severe frontal hypometabolism has been correlated with an increased severity of cognitive and behavioural impairments [13]. Besides group differences between ALS patients with or without cognitive impairments, correlations have been described between metabolism in specific brain regions and performance in cognitive tasks. An impaired cognitive theory of mind has been linked to increased metabolism in the left fusiform gyrus [10], and decreased metabolism in the prefrontal cortex [14], the supplementary motor area [14], and the left inferior frontal gyrus [15]. Weaker episodic memory performance has been associated with increased metabolism in the hippocampus and left parahippocampal gyrus [10]. Concerning behavioural impairment, the increasing severity of apathy has been negatively associated with metabolism in the prefrontal cortex, premotor cortex, anterior cingulate gyrus, and insula as well as positively associated with metabolism in the cerebellum and pons [16]. However, evidence is still scarce on associations between glucose metabolism and specific cognitive domains relevant in ALS such as executive functioning, verbal fluency, and social cognition.

An important consideration for PET analyses are partial volume effects (PVEs). The radiotracer signal measured in a given brain region reflects a combination of the true regional signal and the signal from neighbouring tissues or CSF, leading to blurring [17]. When the signal in grey matter is higher than in white matter or CSF, as is the case with FDG-PET, PVEs lead to an underestimation of the true grey matter signal. Besides scanner resolution, the extent of PVEs is further dependent on the size of the measured region and the presence of atrophy [17]. The majority of FDG-PET studies did not include a PVE correction (PVEC) [8, 9, 11, 13, 16] based on the assumption that metabolic measurements in ALS are relatively unaffected by PVEs, but this assumption has not yet widely been tested.

The aims of this study were thus (1) to replicate findings on differences in glucose metabolism and grey matter volume between ALS patients with and without cognitive or behavioural impairment, (2) to investigate associations of glucose metabolism and grey matter volume with performance in ALS-related cognitive domains, and (3) to examine the impact of PVEC of the FDG-PET data on the group differences and domain-specific associations.

## Materials and methods

### Participants

67 patients were recruited by the Department of Neurology of the Rostock University Medical Centre. They were diagnosed according to the modified El Escorial criteria [18] and characterised using the revised ALS Functional Rating Scale (ALSFRS-R) [19]. The exclusion criteria included a history of brain injury, epilepsy, psychiatric illness, or a diagnosis of dementia [20].

### Neuropsychological assessment

The neuropsychological assessment included the German versions of the Edinburgh cognitive and behavioural ALS screen (ECAS) [21], Faux Pas test [22], the Facial Recognition Task [23], and the informant version of the Frontal System Behaviour Scale (FrSBe) [24]. ECAS assesses five cognitive domains with multiple subtests [25], the Faux-pas test is an advanced theory of mind task, and the Facial Recognition Task measures the ability to identify the six basic emotions based on facial expressions. The FrSBe assesses behavioural changes in apathy, disinhibition, and executive dysfunction.

Cognitive and behavioural impairments were classified according to the revised Strong criteria [26] as follows: patients without cognitive and/or behavioural impairment (ALSni), patients with cognitive impairment (ALSci), patients with behavioural impairment (ALSbi) and patients with cognitive and behavioural impairment (ALScbi). Cognitive impairment was defined as a deficient score in the verbal fluency, executive functions, or language domain based on age- and education-adjusted ECAS-norms from a Swiss and German sample [21]. Norms from a French sample were used for Faux Pas test and Facial Recognition Test [27]. The behavioural classification was based on either or both the age- and education-normed informant version of FrSBe [24], and the clinical observation of an experienced neuropsychologist.

### Neuroimaging data acquisition

Structural MRI scans were taken on two scanners and FDG-PET scans were acquired on the same scanner at the Rostock University Medical Centre (Rostock, Germany). High-resolution T1-weighted anatomical images were obtained on a 3 T Siemens Skyra Fit scanner (2018–2022) or on a Siemens Vida scanner (from 2022 onwards) using the magnetization-prepared rapid gradient echo (MPRAGE) sequence with the following parameters: 192 sagittal slices,

field of view = 256 × 256 $mm^2$, image matrix = 256 × 256, isotropic voxel size = 1 $mm^3$, echo time = 4.37 ms, repetition time = 2500 ms, and flip angle = 7°.

The patients underwent dynamic PET imaging (4 × 5 min) of the brain using a Gemini TF 16 scanner (Philips Healthcare) at 30 min after the injection of 199 ± 18 MBq FDG. Prior to PET imaging, an auxiliary CT scan (120 kVp, 30 mAs) was performed. To minimise the influence of motion artefacts, the four dynamic PET frames were examined for possible head movements of the patient. Conspicuous frames were excluded, and finally, a static PET data set, representing at least 10 min acquisition time, was reconstructed using the manufacturer's proprietary BLOB-OS reconstruction algorithm (3 iterations, 31 subsets) corrected for randoms, scatter, decay, and attenuation using information from the auxiliary CT.

## Neuroimaging preprocessing

All imaging data were preprocessed in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/) using Matlab.

*MRI:* The MRI scans were skull-stripped, segmented, and spatially normalised to the MNI152 non-linear asymmetric 2009 template using Dartel registration in the CAT12 toolbox [28]. The grey matter (GM), white matter (WM), and CSF segments in addition to the forward and inverse deformation fields were saved. The scans were smoothed with a 8 mm kernel and submitted to statistical analysis. In addition, an explicit GM mask was created by keeping GM voxels that overlap in at least 50% of the spatially normalised scans and applied in all statistical analyses.

*FDG-PET:* The FDG-PET scan was coregistered to the participant's MRI scan and preprocessed both with and without PVEC according to the Müller-Gärtner [29] method using custom Matlab scripts to enable a comparison of results. GM, WM, and CSF segments from the MRI preprocessing were smoothed with a 4.8 mm kernel, accounting for the spatial resolution of the used PET-system [30], and used in the PVEC procedure. A GM threshold of 0.5 was incorporated, and trilinear interpolation was used. For the PVEC procedure, WM and CSF signal was estimated for each individual patient by thresholding their WM and CSF segments at 0.99 and averaging the signal in the resulting mask. The output of PVEC was a map of corrected FDG signal restricted to GM. Negative voxels were masked-out, and to compare results with and without PVEC the negative and non-GM voxels were also masked-out in the non-corrected FDG-PET (NC-FDG) scans. Intensity normalization on both PVEC-FDG and NC-FDG scans was carried out by dividing the signal of each voxel by the average FDG signal within GM [13, 15, 16]. This intensity normalisation approach impacts the interpretation of hypermetabolism: when global metabolism is reduced throughout widespread hypometabolic regions, then clusters of hypermetabolism may instead indicate a relative preservation of metabolism. Subsequently, all scans were spatially normalised to MNI space by applying forward deformation fields from MRI preprocessing step with trilinear interpolation. All scans were smoothed with a 10 mm kernel and submitted to statistical analysis.

## Statistical analysis

The whole sample was characterised by various demographic and clinical data and equivalence of the groups was compared using Chi-squared tests for categorical variables and Analysis of Variance (ANOVA) for continuous variables in the Statistical Product and Service Solutions (SPSS, version 28.0). Kruskal–Wallis tests were used to compare the groups on individual cognitive domains. For cognitive domains where the groups differed, individual subtest scores contributing to the domain score were also compared. Mann–Whitney U tests were used for all post hoc comparisons.

Group differences in PVEC-FDG data, NC-FDG data, and GM volume were tested in a voxel-based manner with one-way ANOVA in SPM12 using Matlab. Age and sex were included as covariates in all analyses, and the total intracranial volume (TIV) was included in GM volume analysis. All possible t-contrasts were tested. Further voxel-based regression analyses on the PVEC-FDG signal, NC-FDG signal, and GM volume were specified in SPM12 for single cognitive domains and subtest scores that differed in the preceding neuropsychology analyses. Age, sex, and years of formal education were included as covariates, and the total intracranial volume (TIV) was included in GM volume analysis.

In addition, corresponding analyses were carried out using partial least squares (PLS) regression with the PLS application in Matlab [31]. This method makes use of covariance between voxels and yields latent variables (LVs) that each identify a pattern of brain regions that covary with some external measure [32]. The significance of each LV was determined with 100 permutation tests at a threshold of $p < 0.05$. Each voxel has a salience on each LV. To determine the reliability of these voxel saliences, we used the bootstrap estimation with 100 iterations and a threshold of $p < 0.05$ for the resulting bootstrap ratios. We examined all group contrasts in addition to the associations with performance on cognitive domains that differed in the preceding neuropsychology analyses using the PVEC-FDG and NC-FDG data.

## Results

### Demographic and clinical characteristics

The clinical and demographic information for our sample can be found in Table 1. Based on the small group size of ALSbi ($n=5$), they were excluded from the group analysis. The equivalence of the groups on different variables was tested using Chi-squared tests for categorical and ANOVAs for quantitative variables.

### Neuropsychological data

The neuropsychological data are summarised in Table 2. The three groups were compared using Kruskal–Wallis and Mann–Whitney tests. For single cognitive domains where the groups differed, subtest scores were also compared. The ALSni and ALSci patients differed on the cognitive domains of language, executive functions, verbal fluency, and memory, whereas differences between ALSni and ALScbi patients were observable on executive functions, verbal fluency, and memory. No differences in cognitive scores were observed between ALSci and ALScbi patients.

### Group analyses

Groups were compared on GM volume, PVEC-FDG data, and NC-FDG data in a voxel-based manner. The height threshold was set at $p<0.001$, $p<0.05$ FWE-corrected at cluster level. If no clusters were detected, an exploratory analysis at a lenient threshold of $p<0.001$ and a minimum cluster size of 100 voxels was undertaken (supplementary Fig. 1). The FWE-corrected results are reported below and both FWE-corrected and exploratory findings can be found in Tables 3 and 4. In addition, PLS analyses of the contrasts were carried out.

#### ALSni vs ALSci

No clusters were detected in GM volume, PVEC-FDG, nor the NC-FDG analysis. No differences could be detected in PLS analyses with both PVEC-FDG data (LV = 93.88, $p=0.574$) and NC-FDG data (LV = 92.18, $p=0.614$).

**Table 1** Clinical and demographic characteristics

| | ALSni | ALSci | ALScbi | Total | *p*-value |
|---|---|---|---|---|---|
| Group size: count (%) | 30 (48.4%) | 21 (33.9%) | 11 (17.7%) | 62 (100%) | **0.013** |
| Age: M (SD) | 62.1 (12.9) | 69.9 (7.9) | 67.3 (9.8) | 65.7 (11.3) | **0.045** |
| Sex: female/male | 9/21 | 10/11 | 2/9 | 21/41 | 0.204 |
| Education years: M (SD) | 14.6 (2.9) | 13.0 (2.4) | 13.4 (2.8) | 13.8 (2.8) | 0.106 |
| Months since onset: median (IQR) | 14 (9.0–24.8) | 9 (6.0–19.0) | 13 (4.5–27.5) | 12.5 (7.0–24.0) | 0.337 |
| Site of onset: count (%) | | | | | 0.482 |
| Spinal onset | 24 (80.0%) | 14 (66.7%) | 9 (81.8%) | 47 (75.8%) | |
| Bulbar onset | 6 (20.0%) | 7 (33.3%) | 2 (18.2%) | 15 (24.2%) | |
| Phenotype: count | | | | | 0.907 |
| Classical ALS | 23 | 15 | 9 | 47 | |
| PLS | 1 | 2 | 0 | 3 | |
| UMND | 2 | 2 | 1 | 5 | |
| PMA | 2 | 1 | 1 | 4 | |
| LMND | 2 | 0 | 0 | 2 | |
| Not classifiable | 0 | 1 | 0 | 1 | |
| ALSFRS-R: median (IQR) | 39.0 (36.3–41.8) | 39.0 (36.0–40.0) | 35.0 (33.0–41.0) | 39.0 (35.0–41.0) | 0.460 |
| Genetic status (count) | | | | | |
| C9ofr72 | 2 | 1 | 1 | 4 | |
| TARDBP | 0 | 1 | 0 | 1 | |
| SOD1 | 0 | 0 | 1 | 1 | |
| No mutation | 15 | 9 | 5 | 29 | |
| Untested | 13 | 10 | 4 | 27 | |

*ALSni* not impaired; *ALSci* cognitively impaired; *ALScbi* cognitively and behaviourally impaired; *M* mean; *SD* standard deviation; *IQR* interquartile range; *PLS* primary lateral sclerosis; *UMND* upper motor neuron-dominant ALS; *PMA* progressive muscular atrophy; *LMND* lower motor neuron-dominant ALS

Table 2 Neuropsychological results

| Dependent variable | Test statistic (*p*-value)[1] | ALSni vs ALSci | | ALSni vs ALScbi | | ALSci vs ALScbi | |
|---|---|---|---|---|---|---|---|
| | | Test statistic (*p*-value)[2] | Effect size[3] | Test statistic (*p*-value)[2] | Effect size[3] | Test statistic (*p*-value)[2] | Effect size[3] |
| ECAS: Language | **8.2 (0.016)** | **180 (0.008)** | 0.373 | 102 (0.053) | 0.302 | 107 (0.732) | 0.060 |
| Naming | 3.4 (0.182) | | | | | | |
| Comprehension | 3.7 (0.156) | | | | | | |
| Spelling | **9.2 (0.010)** | **209.5 (0.026)** | 0.311 | **76.5 (0.004)** | 0.444 | 98.5 (0.490) | 0.122 |
| ECAS: Executive functions | **15.8 (0.001)** | **135 (0.001)** | 0.483 | **64 (0.003)** | 0.466 | 106 (0.704) | 0.054 |
| Digit span | **8.0 (0.019)** | **213.5 (0.049)** | 0.275 | **82 (0.013)** | 0.388 | 87.5 (0.255) | 0.201 |
| Alternation | **8.2 (0.016)** | **199 (0.015)** | 0.342 | **92 (0.017)** | 0.372 | 114.5 (0.967) | 0.007 |
| Inhibition | **7.4 (0.024)** | **193 (0.017)** | 0.335 | **99 (0.045)** | 0.313 | 102 (0.583) | 0.097 |
| Social cognition | **14.0 (0.001)** | **196 (0.002)** | 0.444 | **97 (0.002)** | 0.477 | 110 (0.810) | 0.043 |
| ECAS: Verbal fluency | **35.1 (0.001)** | **34 (0.001)** | **0.878** | **31.5 (0.001)** | **0.621** | 97 (0.450) | 0.133 |
| S-words | **27.7 (0.001)** | **541.5 (0.001)** | **0.607** | **311 (0.001)** | **0.671** | 111.5 (0.874) | 0.028 |
| G-words | **30.0 (0.001)** | **578 (0.001)** | **0.705** | **286.5 (0.001)** | **0.558** | 86.5 (0.248) | 0.204 |
| ECAS: Memory | **10.6 (0.005)** | **163.5 (0.004)** | 0.407 | **88 (0.023)** | 0.355 | 97 (0.460) | 0.130 |
| Immediate recall | **13.6 (0.001)** | **156 (0.002)** | 0.430 | **69 (0.004)** | 0.446 | 92 (0.344) | 0.167 |
| Delayed recall | **11.4 (0.003)** | **161 (0.003)** | 0.418 | **82.5 (0.014)** | 0.384 | 100 (0.533) | 0.110 |
| Recognition | 5.2 (0.075) | | | | | | |
| ECAS: Visuo-spatial | 0.6 (0.732) | | | | | | |
| Faux Pas test | **8.2 (0.017)** | **55.5 (0.024)** | 0.405 | **36 (0.025)** | 0.424 | 29.5 (0.230) | 0.275 |
| Facial recognition task | 3.6 (0.165) | | | | | | |
| FrSBe: total | **6.9 (0.031)** | 54.5 (0.616) | 0.112 | **63 (0.010)** | **0.598** | **39.5 (0.045)** | **0.548** |
| Apathy | **9.1 (0.010)** | 50 (0.877) | 0.035 | **67 (0.004)** | **0.685** | **42.5 (0.017)** | **0.639** |
| Disinhibition | 2.2 (0.340) | | | | | | |
| Executive dysfunction | 4.9 (0.87) | | | | | | |

*ALSni* not impaired; *ALSci* cognitively impaired; *ALScbi* cognitively and behaviourally impaired. [1]*H* statistic of the Kruskal–Wallis Test with 3 groups (asymptotic 2-tailed significance), [2]*U* statistic of the Mann–Whitney Test (asymptotic 2-tailed significance), [3]Pearson *r*

*P*-values < 0.05 and effect sizes > 0.5 are bold

### ALSni vs ALScbi

For PVEC-FDG and NC-FDG data, ALScbi patients showed hypometabolic clusters in the left superior frontal gyrus and right temporal pole. In PVEC-FDG analyses, the ALScbi group further showed hypometabolism in the right middle-frontal gyrus, and hypermetabolism in right superior occipital gyrus. The clusters are listed in Table 3 and portrayed on Fig. 1. The PLS analyses corroborated these results by identifying a similar metabolic pattern in both PVEC-FDG data (LV = 135.69, $p < 0.001$) and NC-FDG data (LV = 135.72, $p < 0.001$).

### ALSci vs ALScbi

In PVEC-FDG and NC-FDG analyses, the ALScbi group showed a cluster of relative hypermetabolism in the left cuneus. The clusters are listed in Table 4 and portrayed on Fig. 1. In the PLS analyses, the difference was on the threshold of significance based on both the NC-FDG data (LV = 104.61, $p = 0.050$) and the PVEC-FDG data (LV = 99.71, $p = 0.059$).

Table 3 Clusters of hypo- and hypermetabolism in ALScbi compared to ALSni patients

| Data | Label | Side | *K* | *x* | *y* | *z* | *P* (uncorr. at cluster level) | *P* FWE (corr. at cluster level) |
|---|---|---|---|---|---|---|---|---|
| Hypometabolism | | | | | | | | |
| PVEC-FDG | Middle frontal gyrus | *R* | 5212 | 38 | 33 | 46 | **0.000** | **0.000** |
| PVEC-FDG | Superior frontal gyrus | *L* | 1214 | – 10 | 52 | 26 | **0.006** | **0.034** |
| PVEC-FDG | Temporal pole | *R* | 1143 | 44 | 20 | -33 | **0.007** | **0.041** |
| PVEC-FDG | Supramarginal gyrus | *R* | 504 | 58 | – 36 | 42 | 0.058 | 0.283 |
| PVEC-FDG | Triangular inferior frontal gyrus | *R* | 264 | 44 | 38 | 3 | 0.157 | 0.594 |
| NC-FDG | Superior frontal gyrus | *R* | 4943 | 9 | 68 | 18 | **0.000** | **0.000** |
| NC-FDG | Temporal pole | *R* | 1443 | 44 | 20 | -34 | **0.003** | **0.016** |
| NC-FDG | Superior frontal gyrus | *L* | 1109 | – 22 | 62 | 0 | **0.007** | **0.042** |
| NC-FDG | Superior frontal gyrus | *L* | 447 | – 10 | 52 | 26 | 0.068 | 0.329 |
| NC-FDG | Triangular inferior frontal gyrus | *R* | 424 | 52 | 38 | 9 | 0.074 | 0.355 |
| NC-FDG | Supramarginal gyrus | *R* | 367 | 58 | – 38 | 42 | 0.094 | 0.427 |
| Hypermetabolism | | | | | | | | |
| PVEC-FDG | Superior occipital gyrus | *R* | 2253 | 20 | – 87 | 20 | **0.000** | **0.003** |
| PVEC-FDG | Cerebellar lobule VI | *R* | 746 | 26 | – 72 | – 18 | **0.025** | 0.133 |
| PVEC-FDG | Cerebellar lobule VI | *L* | 623 | – 32 | – 69 | – 20 | 0.038 | 0.194 |
| PVEC-FDG | Middle occipital gyrus | *L* | 321 | – 34 | – 94 | – 6 | 0.122 | 0.502 |
| PVEC-FDG | Superior occipital gyrus | *L* | 226 | – 16 | – 88 | 18 | 0.189 | 0.661 |
| NC-FDG | Cerebellar lobule VI | *R* | 586 | 26 | – 72 | – 18 | **0.040** | 0.209 |
| NC-FDG | Fusiform gyrus | *L* | 496 | – 36 | – 70 | – 16 | 0.056 | 0.281 |
| NC-FDG | Superior occipital gyrus | *R* | 412 | 16 | – 92 | 20 | 0.078 | 0.369 |
| NC-FDG | Cuneus | *L* | 406 | – 9 | – 92 | 14 | 0.080 | 0.377 |
| NC-FDG | Superior occipital gyrus | *L* | 214 | – 16 | – 87 | 18 | 0.192 | 0.679 |
| NC-FDG | Middle occipital gyrus | *L* | 213 | – 36 | – 94 | – 8 | 0.193 | 0.681 |

*K* cluster size in voxels; *FWE* family wise error; *PVEC-FDG* PVE-corrected FDG-PET data; *NC-FDG* non-corrected FDG-PET data; *R* right; *L* left. Only clusters with more than 200 voxels are presented

Table 4 Clusters of hypo- and hypermetabolism in ALScbi compared to ALSci patients

| Data | Label | Side | *K* | *x* | *y* | *z* | *P* (uncorr. at cluster level) | P FWE (corr. at cluster level) |
|---|---|---|---|---|---|---|---|---|
| Hypometabolism | | | | | | | | |
| PVEC-FDG | Temporal pole | R | 500 | 42 | 21 | – 33 | 0.059 | 0.287 |
| PVEC-FDG | Superior frontal gyrus | R | 469 | 33 | 62 | 20 | 0.066 | 0.317 |
| NC-FDG | Superior frontal gyrus | R | 509 | 33 | 60 | 21 | 0.053 | 0.269 |
| NC-FDG | Temporal pole | R | 502 | 40 | 22 | – 34 | 0.054 | 0.275 |
| Hypermetabolism | | | | | | | | |
| PVEC-FDG | Cuneus | L | 1088 | – 8 | – 87 | 15 | **0.009** | **0.048** |
| NC-FDG | Cuneus | L | 1460 | – 8 | – 87 | 15 | **0.003** | **0.016** |

*K* cluster size in voxels; *FWE* family wise error; *PVEC-FDG* PVE-corrected FDG-PET data; *NC-FDG* non-corrected FDG-PET data; *R* right; *L* left. Only clusters with more than 200 voxels are presented

## Associations with single cognitive domains and subtests

In the PVEC-FDG and NC-FDG analyses, no clusters were detected with FWE-correction at cluster level. Further exploratory analyses at a height threshold of $p < 0.001$ and a minimum cluster size of 100 voxels identified the following associations between glucose metabolism and single cognitive domains and their subtests (Fig. 2). All correlations were positive unless otherwise specified. The domain score of executive functioning correlated with metabolism in the left middle-frontal gyrus (NC-FDG), whereas its

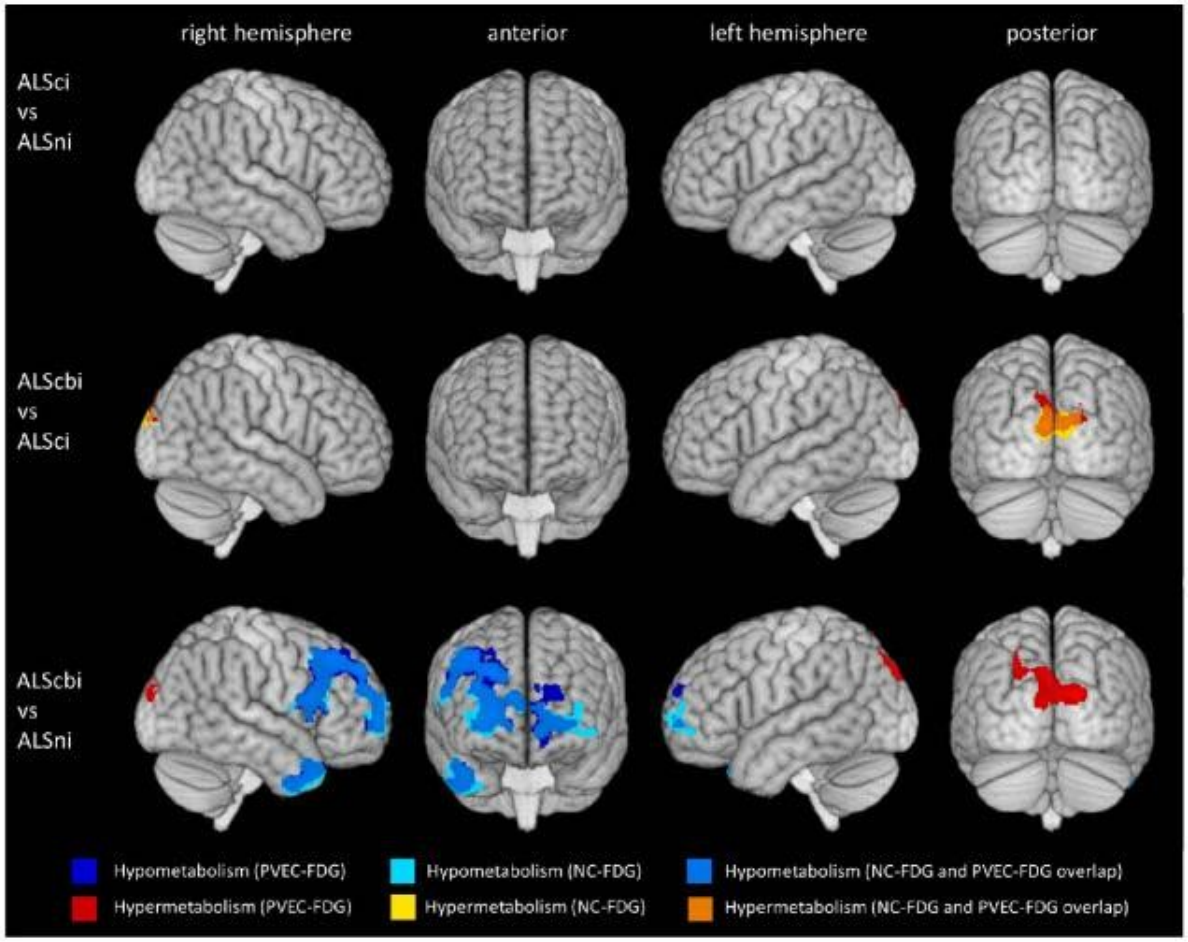


**Fig. 1** Clusters of relative hypo- and hypermetabolism per group comparison. The FDG-PET signal is relative to the average FDG-tracer uptake in GM. Height threshold is $p<0.001$ and all data are $p<0.05$ FWE-corrected at cluster level. *ALSni* not impaired; ALSci, cognitively impaired; *ALScbi* cognitively and behaviourally impaired; *PVEC-FDG* PVE-corrected FDG-PET data; *NC-FDG* non-corrected FDG-PET data

subtest inhibition correlated with left lingual gyrus metabolism (NC-FDG), and the subtest alternation with metabolism in the left lobule VIII of the cerebellum (NC-FDG), the left precentral gyrus (PVEC-FDG), and the left middle temporal gyrus (PVEC-FDG). The verbal fluency subtest of S-words correlated with metabolism in the bilateral superior frontal (PVEC-FDG and NC-FDG), left precentral (PVEC-FDG), and right inferior orbital gyrus (NC-FDG). Furthermore, negative associations were observed between the S-words score and metabolism in the bilateral crus I of the cerebellum. Memory performance was associated with metabolism in the right precuneus (PVEC-FDG), and the left fusiform gyrus (PVEC-FDG and NC-FDG) and the subtest of immediate recall was associated with the right precuneus (PVEC-FDG and NC-FDG) and the left middle temporal gyrus metabolism (PVEC-FDG and NC-FDG). Faux Pas test scores correlated with metabolism in the right middle-frontal gyrus (PVEC-FDG and NC-FDG) and right precentral gyrus (PVEC-FDG). None of the PLS analyses with PVEC-FDG or NC-FDG data neared significance (data not shown). Clusters of GM volume are outlined in supplementary Table 1.

## Discussion

The aim of this study was to investigate the associations between cerebral glucose metabolism and cognition in ALS using group comparisons and regression analyses. In accordance with previous work in the field [13], we found that concurrent cognitive and behavioural impairment were associated with widespread changes in glucose metabolism.

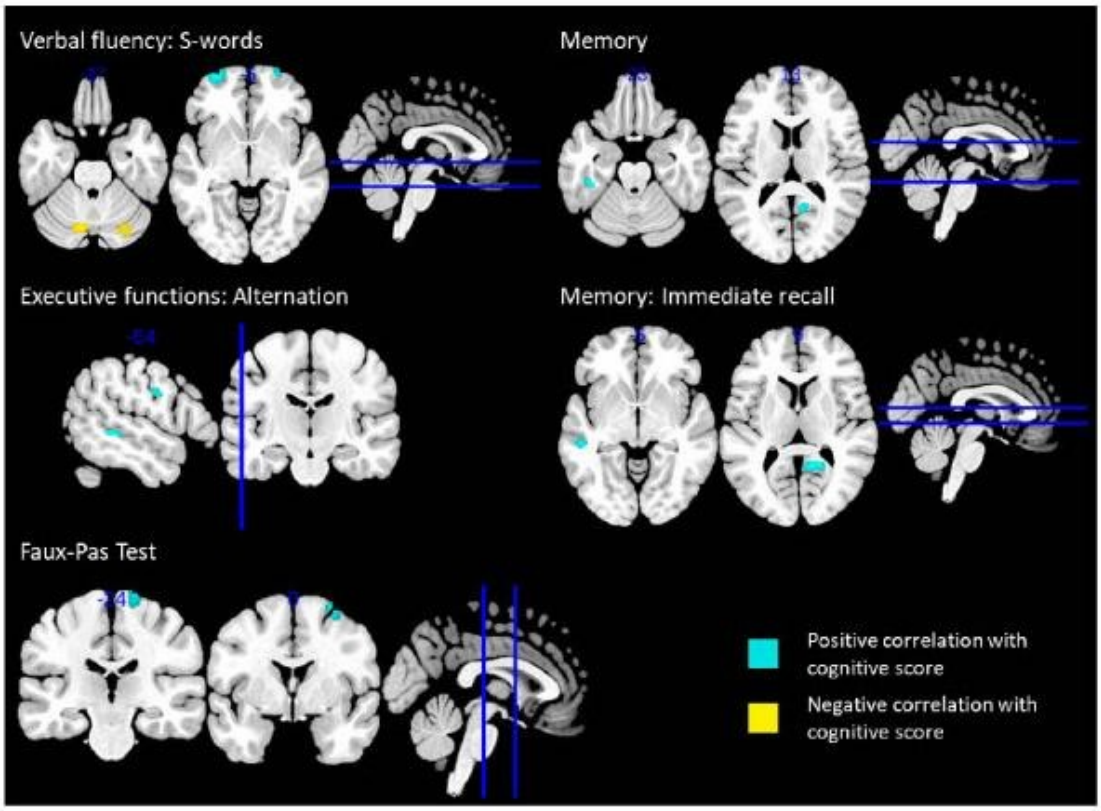


Fig. 2 Correlations of glucose metabolism and cognitive scores. The FDG-PET signal is relative to the average FDG-tracer uptake in GM. Height threshold is $p<0.001$ and minimum cluster size is 100 voxels

Relative to the average cortical FDG-PET signal, hypometabolism in the superior frontal gyri, middle-frontal gyri, and temporal pole in addition to relative hypermetabolism in the superior occipital gyrus was detected in ALScbi when compared to ALSni. In exploratory analyses without FWE-correction, the comparison of ALScbi to ALSci identified a similar pattern of metabolism changes where the location of the clusters persisted, but their extent was conspicuously limited. This pattern did not emerge for the group comparison of ALSci to ALSni, even in the exploratory analysis without FWE-correction. The result of the partial least squares (PLS) analysis further corroborated these group comparison findings. The FDG-PET signal was intensity normalised using the average FDG signal in GM, which impacts the interpretation of hypermetabolic clusters. In the case of widespread hypometabolic areas leading to a reduction in global metabolism, the co-occurring clusters of hypermetabolism should be interpreted as a relative preservation of metabolism.

In contrast to both our expectations and a previous publication [13], no clusters of hypometabolism survived the FWE-correction in ALSci compared to ALSni patients in the current sample. One possible explanation for this lack of stark differences may be the heterogeneous composition of the ALSci as defined by Strong [26], since this category includes patients with mild deficits in either verbal fluency, executive functioning, or language, and those impairments may have different neural substrates. A further consideration is the use of the ECAS [25] instead of a comprehensive neuropsychological test battery (owing to both its feasibility and its lower burden for patients). The prevalence of deficits in ALS-specific cognitive functions (fluency, executive, and language) in our sample was comparable to the original publication, which was based on and validated in UK samples [25]. However, recent research has highlighted how crucial the choice of population norms and statistical methods are in determining the prevalence of cognitive impairment based on the ECAS [33]. The results of the group comparisons in our sample are, therefore, inherently dependent on the available age- and education-adjusted German ECAS-norms [21]. This raises questions of eligibility to directly compare our results with other cohorts and has implications for the generalisation of our findings.

As would be expected, ALSni had higher test scores than both ALSci and ALScbi groups in the majority of cognitive domains, and our sample showed no significant differences in cognitive scores between ALSci and ALScbi. Behavioural changes were assessed with the FrSBe test [24], which comprises three domains: apathy, disinhibition, and executive dysfunction. As anticipated, only the ALScbi group differed from ALSni and ALSci on the total score and the apathy subscale of the test. These neuropsychological results provide context for interpreting the glucose metabolism findings. The explorative non-FWE-corrected comparison of ALSci to ALSni revealed one relatively small hypometabolic cluster in the precentral gyrus despite significant differences between the groups in the majority of assessed cognitive domains. Furthermore, the explorative non-FWE-corrected comparison of ALScbi to ALSci showed a similar distribution of hypo- and hypermetabolic clusters as the FWE-corrected ALScbi-ALSni comparison despite a lack of group differences between ALScbi and ALSci on cognitive scores. Alterations in glucose metabolism may thus be more closely related to the presence of behavioural changes than mild cognitive impairment. However, as behavioural changes cannot be completely separated from cognitive changes, particularly changes in inhibitory control and executive functioning, this somewhat limits the interpretation of our results. Further investigations are required to assess the comparative effects of glucose metabolism on cognitive and behavioural symptoms in ALS.

We observed significant differences in ALScbi when compared to ALSni regarding hypometabolism: the clusters were strongly asymmetric, and largely restricted to the frontal and temporal lobes of the right hemisphere. The notion that impaired behaviour is associated with changes in cerebral glucose metabolism has also been demonstrated by Canosa et al. [16]. Though in contrast to our markedly right hemispheric clusters, their findings showed bihemispheric clusters of negative correlation between glucose metabolism and apathy as determined by a subscale of FrSBe. Whereas their study focussed specifically on correlations of apathy, our study considered behavioural impairment in a more generalised manner. The right lateralisation of hypometabolism in behaviourally impaired ALS in this study is in accord with a tendency for right hemispheric dominance in emotional functioning in the neurobiological literature [34].

Concerning voxel-wise analyses of FDG-PET data respective to individual cognitive domains and their subtests, numerous positive correlations could be observed in the explorative SPM analyses without FWE-correction (height threshold of $p < 0.001$, a minimum cluster size of 100 voxels), whereas corresponding analyses utilising the PLS approach indicated a lack of associations. The diverging nature of these findings suggests an absence of strong correlations between cognitive performance and glucose metabolism at rest. Furthermore, only explorative analyses at a lenient threshold yielded associations with GM volume, which is in agreement with a previous publication [35] that reported small clusters of correlations between GM volume and ECAS scores. Therefore, possible associations between cognition and FDG-PET and GM, if present, seem to be weak.

Several studies reported applying a PVEC [10, 14, 15] and others did not [8, 9, 11, 13, 16]. Here, we directly compared the results of FDG-PET analyses with and without PVEC. The analyses with and without PVEC led to similar results. The clusters identified with both approaches overlapped to a large degree, and the LVs and their significance in the PLS analyses were similar. The influence of PVEs on FDG-PET signals seems to be negligible in ALS samples, and carrying out a PVEC does not seem to affect the results.

We need to consider several limitations of this study. First, a larger sample size would provide higher statistical power to better detect small effects and to give more confidence in interpreting negative findings as absence of true effects. Furthermore, a comprehensive neuropsychological test battery and/or population norms based on larger samples could have yielded more accurate categorisations between the cognitive groups in addition to a more detailed assessment of individual cognitive domains in contrast to the ECAS assessment [10, 13, 15, 36]. Unfortunately, the impact of motor symptoms on test performance cannot be controlled for many cognitive tests and therefore limits the validity of such test findings in ALS patients. The ECAS is able to assess cognition independent of the severity of motor symptoms and has therefore been adapted in the routine assessment of ALS patients in our centre as well as many others. Although the ECAS has become a standard screening instrument for cognitive assessment in ALS owing to its practicability and feasibility placing far less burden on patients with the shorter testing time, our findings suggest that utilising the ECAS for research purposes is less desirable than in clinical routine.

In conclusion, we found that concurrent cognitive and behavioural impairment in ALS was associated with widespread changes in glucose metabolism. In contrast to previous results which suggested notable differences in glucose hypometabolism between ALSni and ALSci groups, in our study, significant differences were only observed in the group comparison between ALSni and ALScbi. In addition, PVEC did not have a conspicuous effect on FDG-PET analyses in an ALS sample, which suggests that the results from publications with and without PVEC are comparable. The individual cognitive domains did not strongly correlate with glucose metabolism or GM volume, but this result should be replicated in larger samples with more comprehensive cognitive assessments.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00415-024-12388-z.

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**Author contributions** Manuscript concept and design: AL, ST, JP. Funding acquisition: AH, BJK, JP. Data collection: EK, JK, BJK, JP. Data analysis and interpretation: AL, JS, EK, ST, JP. Manuscript drafting: AL. Manuscript revision and approval of the final version: all.

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**Data availability** The data analysed in the current study can be received from the corresponding author upon reasonable request from qualified investigators.

## Declarations

**Conflicts of interest** S.T. served on advisory boards of Lilly, Eisai, and Biogen, and is member of the independent data safety and monitoring board of the ENVISION study (Biogen).

**Ethical approval** The study was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients gave their written informed consent, and the local ethics committee at the Rostock University Medical Centre approved the study (2022–0081).



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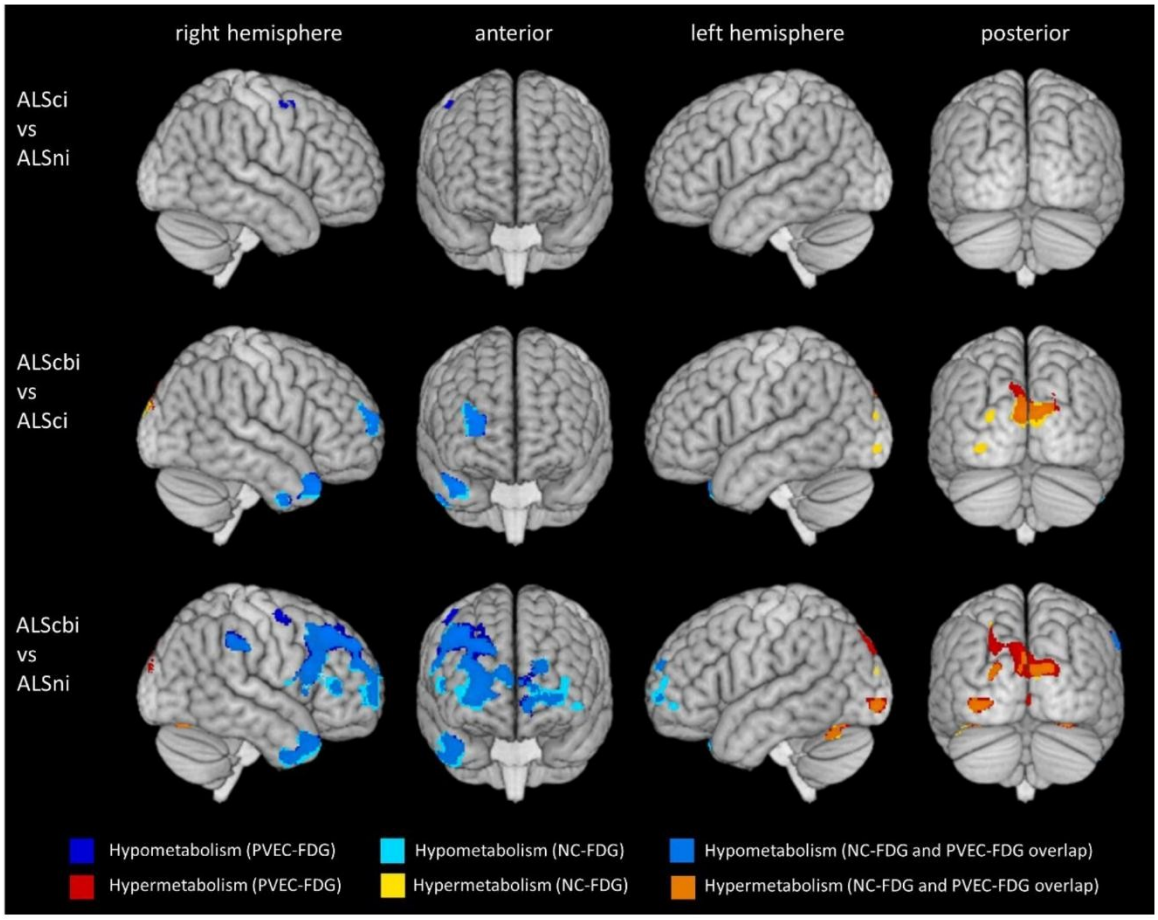
## Supplementary material

**Supplementary Figure 1**. Clusters of relative hypo- and hypermetabolism per group comparison




The FDG-PET signal is relative to the average FDG-tracer uptake in GM. Height threshold is $p<0.001$ and minimum cluster size is 100 voxels for all data.

ALSni, not impaired; ALSci, cognitively impaired; ALScbi, cognitively and behaviourally impaired; PVEC-FDG, PVE-corrected FDG-PET data; NC-FDG, non-corrected FDG-PET data.

**Supplementary Table 1.** Clusters of GM volume associated with cognitive domain scores.

| Labels | Side | K | x | y | z | P (uncorr. at cluster level) | P FWE (corr. at cluster level) |
|---|---|---|---|---|---|---|---|
| ECAS: Executive functions | | | | | | | |
| Cerebellum VIII | R | 1006 | 10 | -72 | -45 | **0.023** | 0.100 |
| Cerebellum Crus II | R | 207 | 18 | -75 | -38 | 0.269 | 0.709 |
| ECAS: Memory | | | | | | | |
| Gyrus rectus | R | 581 | 9 | 36 | -28 | 0.072 | 0.284 |
| Cuneus | R | 317 | 15 | -74 | 30 | 0.173 | 0.550 |
| Precuneus | L | 242 | -21 | -64 | 16 | 0.231 | 0.656 |
| Hippocampus | L | 201 | -32 | -24 | -12 | 0.278 | 0.724 |

FWE = family wise error, K = cluster size in voxels, R = right, L = left. Only clusters with more than 200 voxels are presented.

## 7.2. Loss of the ipsilateral silent period in amyotrophic lateral sclerosis is associated with reduced white matter integrity in the motor section of the corpus callosum

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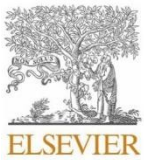

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# Loss of the ipsilateral silent period in amyotrophic lateral sclerosis is associated with reduced white matter integrity in the motor section of the corpus callosum

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Amyotrophic lateral sclerosis
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ABSTRACT

*Objective:* Interhemispheric neurons in the motor section of the corpus callosum have an inhibitory effect on neurons of the contralateral motor cortex. Three quarters of patients with amyotrophic laterals sclerosis (ALS) show impaired transcallosal inhibition. We aimed to investigate whether structural changes co-occur with this functional impairment and to explore its phenotypic correlates.

*Methods:* The demographic, clinical, and neuropsychological data of 127 ALS patients were analysed. Transcallosal inhibition was assessed with an ipsilateral silent period (iSP) protocol using transcranial magnetic stimulation. Patients were categorised based on an iSP response or its loss, and the groups were characterised by demographic, clinical, and neuropsychological variables. Diffusion-weighted images from a subset of 63 patients were analysed using tractography, and white matter (WM) structural integrity metrics were compared across groups.

*Results:* 54 % of patients displayed iSP loss. The average free-water-corrected fractional anisotropy values within the callosal tract between the primary motor cortices were lower for patients with iSP loss compared to patients with an iSP response. There were no group differences based on other diffusivity metrics. The groups did not differ regarding any of the demographic, clinical, or neuropsychological variables.

*Interpretation:* We found reduced WM integrity in the motor section of the corpus callosum that differentiated ALS patients with iSP loss from patients with an iSP response, but with a small effect size. Nevertheless, the underlying pathological substrate and potential genetic drivers for these structural and functional changes in a subset of ALS patients remain to be satisfactorily investigated.

## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by combined dysfunction of upper and lower motor neurons. ALS has increasingly been recognised as a multisystem disorder with widespread neuropathological changes. The corpus callosum (CC) facilitates communication between the cerebral hemispheres and plays an important role in the coordination of voluntary movement. Patients with a disruption of transcallosal inhibitory signalling display involuntary movements that mirror contralateral voluntary movements, called mirror movements [1,2]. Both neuropathological and diffusion-weighted imaging studies have consistently shown structural changes to CC in ALS [3–6]. The integrity of callosal motor fibres can functionally be assessed by transcallosal inhibition, which is measured by transcranial magnetic stimulation (TMS). Deficits in transcallosal inhibition are common in ALS patients and have been suggested to have a high clinical value as an early diagnostic marker [2,7–11]. In cases with a low diagnostic certainty based on a clinical examination, assessing transcallosal inhibition may offer valuable information [2,9,10].

One measure of transcallosal inhibition is the ipsilateral silent period

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(iSP), which can be measured with a short single-pulse TMS protocol and has been found delayed or absent in 68–81 % of ALS patients, indicative of a reduced functional integrity of transcallosal inhibitory neurons in the motor section of the CC [7,9]. ALS patients with a loss of the iSP show more mirror movements compared with ALS patients with a preserved iSP response and healthy controls [11]. Furthermore, reduction in transcranial inhibition is correlated with a faster rate of disease progression [12]. However, no associations have been found between reduced transcranial inhibition and age [11,12], disease duration [11,12], sex [12], ratings on the revised ALS Functional Rating Scale (ALSFRS-R) [9,12], site of onset [9], or muscle strength in ALS patients [11]. It was our intent to extend these previous findings by utilising a large and representative sample that would enable a well-powered investigation of associations. The aim of this study was to correlate transcallosal inhibition with non-motor functioning in our ALS cohort. We opted to focus on non-motor functioning in the form of cognition and behaviour as cognitive and behavioural changes in ALS patients have been established as prominent non-motor symptoms [13] and have been associated with wide-spread structural changes [14–16]. No study seems to have investigated whether cognitive and behavioural changes are more likely to co-occur with impaired transcallosal inhibition. Furthermore, no previous studies have identified associations of structural integrity changes in white matter (WM) with this functional impairment [7,9].

To address this knowledge gap, we studied a large sample of ALS patients (i) to assess the structural correlates of pathological transcallosal inhibition in the callosal tract between motor cortices, and (ii) to compare the demographic, clinical, and neuropsychological profiles of ALS patients with intact and deficient transcallosal inhibition as assessed by an iSP protocol.

## 2. Methods

### 2.1. Participants

In this single centre retrospective study, we analysed data from 127 patients who were recruited between 01/2011 and 10/2023 by the department of neurology of the Rostock University Medical Centre and completed a transcranial magnetic stimulation (TMS) assessment. Patients were classified according to the modified El Escorial criteria for ALS [17] ($n = 127$). A subsample of 9 cases additionally met the Rascovsky criteria for frontotemporal dementia [18]. All patients received a clinical and neurological examination and were characterised on the ALSFRS-R [19]. Exclusion criteria included a history of brain injury, epilepsy, or psychiatric illness.

### 2.2. Neuropsychological assessment

The neuropsychological assessment included the German versions of the Montreal cognitive assessment (MoCA) [20], the Edinburgh cognitive and behavioural ALS screen (ECAS) [21,22], and the Frontal System Behaviour Scale (FrSBe) [23]. Cognitive and behavioural impairments were classified according to the revised Strong criteria. Cognitive deficits were assessed with ECAS based on norms from a Swiss and German sample [22]. A behavioural classification was based either on the age- and education-normed informant version of FrSBe [23], or on clinical observation by an experienced neuropsychologist, or both.

### 2.3. TMS acquisition

Motor evoked potentials (MEP) were recorded from the abductor pollicis brevis (APB) or the first dorsal interosseous (FDI) muscle of each hand with a circular coil (outside diameter 9 cm) connected to a Magstim 200 stimulator (Magstim Co., UK) in a Micromed EP system (Micromed, Germany). Voluntary electromyography activity was recorded with silver/silver chloride surface electrodes in a belly-tendon montage and monitored by audiovisual feedback. The point of optimal excitability was determined.

ISP was recorded with a 7 cm diameter figure-of-eight coil by stimulating at 1.5 times the resting motor threshold while patients maintained a maximum tonic activation of the ipsilateral muscle and relaxed the contralateral muscle. 10 trials per hemisphere were recorded and the latency was averaged after offline rectification.

Peripheral motor latency was measured using foraminal electromagnetic stimulation with a round coil centered over the C7/C8 cervical spine and the central motor conduction time (CMCT) was calculated by subtracting the shortest recorded peripheral nerve component from the shortest total corticomotor latency. CMCT was considered abnormal if longer than 9 ms [24,25].

### 2.4. MRI acquisition and preprocessing

MRI datasets were available for 63 ALS patients, none of whom had a concurrent dementia diagnosis. Diffusion-weighted imaging data were acquired on two scanners at the Rostock University Medical Centre (Rostock, Germany): a 3 Tesla Siemens Skyra Fit scanner (2018–2022) or a Siemens Vida scanner (2022−2023). The MRI diffusion sequence was applied with the following parameters: repetition time = 12,100 ms, echo time = 88 ms, field of view = 240 mm, isotropic resolution = 2 mm, b-values = 700 and 1000 s/mm$^2$, diffusion directions per b-value = 30, b0 images = 10, slices = 72, phase encoding AP. The data were processed in FSL (www.fmrib.ox.ac.uk/fsl, version 6.0.3). Pre-processing included brain-tissue extraction [26] and correction for eddy currents and motion [27].

$T_1$-weighted MR images were acquired with a magnetization prepared rapid gradient echo sequence with the following parameters: 192 sagittal slices, field of view = 256 × 256 mm$^2$, image matrix = 256 × 256, isotropic voxel size = 1 mm$^3$, echo time = 4.37 msec, repetition time = 2500 msec, flip angle = 7°. The data were segmented with cat12 [28] in SPM12.

### 2.5. Estimation of diffusivity metrics

A bi-tensor model was fitted to the eddy-corrected diffusion data using the Diffusion Imaging in Python algorithm [29], which allowed the estimation of free water fraction (FWf), free water-corrected fractional anisotropy (fwFA), free water-corrected mean diffusivity (fwMD), free water-corrected axial diffusivity (fwAD), and free water-corrected radial diffusivity (fwRD). For comparison purposes, a one-compartment tensor model was fitted to estimate the equivalent non-free-water-corrected maps using FSL's dtifit [30] (FA, MD, AD, RD). The analyses with non-corrected data are included in the supplementary material.

To achieve a higher accuracy of registration within the analyses, an unbiased group template was estimated from the FA images of all patients using Advanced Normalization Tools' (ANTs) buildtemplate function [31]. The transformations from this step were applied to warp the diffusivity maps into group template space for voxel-level SPM analyses.

### 2.6. Regions of interest

Six region of interest (ROI) masks were used to guide the probabilistic tractography. Both the right and the left precentral gyrus upper limb area from the Brainnetome Atlas [32] were used as seed and target regions. To constrain the tractography to the motor section of the CC, the equivalent CC mask from the Brainnetome Atlas [32] was included as the waypoint, and the masks of external capsule, internal capsule, and superior longitudinal fasciculus from the Johns Hopkins University (JHU) WM atlas [33] were used as exclusion masks. All masks were in the JHU WM atlas standard space.

All ROIs were transformed into each individual's $T_2$ space by

applying two transformations in one step. The group template was registered to the mean FA image from the JHU WM atlas. The inverse transformation of this step and the inverse transformation from the group template creation step were applied to warp the ROIs into each patient's $T_2$ space. All transformations were estimated with ANTs' nonlinear SyN registration algorithm [34].

### 2.7. Tractography

The diffusion parameters were calculated with FSL's BedpostX using the eddy-corrected DWI data and a standard ball-and-sticks model with three fibres modelled per voxel [35,36]. FSL's ProbtrackX was used for probabilistic tracking by generating 5000 random samples from the seed ROI [35,36]. Two tractograms were calculated for every patient, starting on the right and left side. The equivalent ROI in the opposite hemisphere was used as the target. Per patient, the two tractograms were combined and transformed into the group template space to estimate a group tract.

The group tract was created by incorporating all voxels that were included in at least 90 % of the individual patient tracts based on visual inspection (Fig. 1). Subsequently, the group tract was transformed back into each patient's $T_2$ space, and average diffusivity metrics were extracted from voxels belonging to the tract for each participant.

To control for unspecific individual differences in diffusivity metrics, a control mask was created for each patient by subtracting the group tract from the patient's whole-brain WM segment (from running cat12 on $T_1$-weighted images). Per diffusivity metric, the mean value of the voxels belonging to the control mask was entered as a covariate in statistical analyses.

### 2.8. Statistical analysis

The statistical analyses were completed in Jeffreys's Amazing Statistics Program (https://jasp-stats.org) and R (https://www.r-project.org/). We selected Bayes factor (BF) hypothesis testing. The calculation of BF enables a quantification of evidence, which may either support or oppose an effect of interest. The data can either support the presence of an effect ($BF_{10} \geq 3$), the absence of an effect ($BF_{10} < 0.33$), or offer inconclusive evidence ($BF_{10}$ between 0.3 and 3). After determining the presence or absence of an effect, the posterior distribution of the plausible standardised parameter values for the estimated effect can be examined. The mean of this posterior distribution gives a point estimate for the parameter value and a credible interval summarises the probability distribution for the plausible parameter values for this effect of interest. The width of the credible interval implies the level of reliability of the estimation, and the inclusion of 0 in the credible interval indicates the plausibility of lack of effect.

The whole sample was categorised based on whether they showed iSP response or its loss. The equivalence of the iSP response vs iSP loss groups was compared on numerous demographic, clinical, and neuropsychological variables. The equivalence of groups on categorical variables was compared utilising Bayesian chi-squared tests (independent multinomial sampling plan), and on standardised continuous variables using Bayesian *t*-tests. For each demographic, clinical, and neuropsychological variable, the presence of an effect was evaluated by inspecting the $BF_{10}$, after which the mean and the 95 % credible interval of the posterior distribution for this estimated effect was determined.

Voxel-wise t-tests of the group tract's diffusivity metrics were carried out between patients with iSP loss versus iSP response in SPM12 in Matlab (https://www.fil.ion.ucl.ac.uk/spm/). To control for unspecific individual differences in WM integrity, the parameter value in whole-brain WM excluding the group tract was included as a covariate. Further covariates included age and sex. The WM diffusivity metrics within the group tract were also compared in volume of interest (VOI) analyses between the groups using Bayesian univariate analysis of covariance (ANCOVA), while controlling for age, sex, and the whole-brain diffusivity metric from the respective control mask. For each diffusivity metric, the $BF_{10}$ for group effect was calculated as well as the mean and the 95 % credible interval of the posterior distribution for the estimated effect.

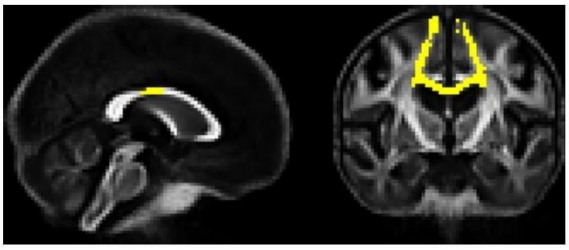

**Fig. 1.** Group tract between the primary motor cortices projected onto the group template.

## 3. Results

54 % of the patients had iSP loss. The iSP response was measured in the APB muscle in 59 patients (46 %) and in the FDI muscle in 68 patients (54 %). The proportion of patients with iSP loss was 64 % when assessing the APB muscle compared to 46 % when assessing the FDI muscle. For patients with an iSP response, the average latency was 43.276 (± 6.517) when measuring from the left hand and 43.954 (± 4.812) when measuring from the right hand. A small proportion of patients showed a loss of iSP on one side and an iSP response on the other side (3 with iSP loss only on the right side, 3 on the left). In seven patients, measurement was only possible on one side due to advanced lower motor neuron damage. CMCT was abnormally slow in 17 % of patients on the left side and in 19 % of patients on the right side.

The demographic and clinical characterisation of the sample can be found below (Table 1). There was moderate evidence that age, sex, phenotypic composition, years of education, and the distribution of Strong classification did not differ between the groups. There was strong evidence that the CMCT on the left side was higher in the iSP loss group than in the iSP response group. Additionally, there was strong evidence that the genetic composition did not differ between the groups ($BF_{10} = 0.040$). In terms of cognitive and behavioural metrics, there was anecdotal to moderate evidence that MoCA score, ECAS total and subscores, FrSBe total and subscores did not differ between the groups (all $BF_{10}$ between 1.8 and 0.2, data shown in supplementary table S1). Despite the sample size of 127 patients, our findings were inconclusive with respect to both the existence ($H_A$) or absence ($H_0$) of group differences between patients with iSP loss and patients with an iSP response on the remaining variables ($BF_{10}$ between 0.3 and 3).

The free-water-corrected data offered strong support that ALS patients with iSP loss had lower fwFA values within the WM tract connecting primary motor cortices ($BF_{10} = 53.741$). The sequential analysis of the level of support ($BF_{10}$ value) for the alternative hypothesis of a group difference with an increasing number of cases is plotted in Fig. 2B. The posterior distribution for the effect of a group was centred at 0.28 (95 % credible interval of 0.11–0.45), indicating a small effect (Fig. 2C). There was no strong evidence for the presence or absence of group differences based on other free-water-corrected diffusivity metrics (Table 2). The equivalent analyses with non-free-water-corrected data can be found in the supplementary material (Table S2). The sequential analysis plot and posterior distribution for fwMD can be found in Fig. 2. Voxel-based analyses of the diffusivity metrics in the group tract did not result in any clusters of group differences at the significance threshold of $p < 0.001$. The figure with non-corrected data is included in the supplementary material (Fig. S1).

**Table 1**
The demographic and clinical characterisation of the sample.

| | N | iSP response | iSP loss | $BF_{10}$ | Mean (95 % CI) |
|---|---|---|---|---|---|
| Group size: count (%) | 127 | 58 (45.7 %) | 69 (54.3 %) | | |
| Muscle: APB/FDI | 127 | 21/37 | 38/31 | 2.036 | 0.182 (0.017, 0.349) |
| Age: mean (SD) | 127 | 64.0 (11.7) | 64.0 (11.1) | 0.190 | 0.002 (−0.172, 0.170) |
| Sex: male/female | 127 | 38/20 | 46/23 | 0.232 | −0.014 (−0.200, 0.163) |
| Education years: mean (SD) | 125 | 13.1 (2.8) | 12.8 (2.5) | 0.225 | −0.049 (−0.221, 0.122) |
| Months since onset: median (IQR) | 118 | 15.0 (7.3–25.0) | 13.0 (7.8–28.0) | 0.385 | 0.102 (−0.078, 0.284) |
| Delta FS: median (IQR) | 113 | 0.7 (0.4–1.2) | 0.7 (0.3–1.4) | 0.417 | 0.109 (−0.075, 0.292) |
| Site of onset: bulbar/spinal | 119 | 19/32 | 17/51 | 0.673 | −0.136 (−0.326, 0.050) |
| Phenotype: count (%) | | | | 0.155 | |
| Classical ALS | | 40 (69 %) | 49 (71 %) | | |
| UMND | | 9 (16 %) | 9 (13 %) | | |
| LMND | | 5 (8 %) | 7 (10 %) | | |
| Not classifiable | 119 | 4 (7 %) | 4 (6 %) | | |
| CMCT: median (IQR) | | | | | |
| left | 107 | 7.3 (6.6–7.9) | 7.7 (6.5–9.2) | 5.987 | 0.238 (0.051, 0.422) |
| right | 108 | 7.2 (6.3–8.3) | 7.6 (6.9–9.1) | 1.588 | 0.185 (0.002, 0.371) |
| Strong criteria: count (%) | 105 | | | 0.128 | |
| ALSni | | 19 (40 %) | 24 (41 %) | | |
| ALSci | | 16 (34 %) | 18 (31 %) | | |
| ALSbi | | 6 (13 %) | 3 (5 %) | | |
| ALScbi | | 6 (13 %) | 13 (22 %) | | |

APB, abductor pollicis brevis muscle; $BF_{10}$, Bayes factor for the presence of group difference; CI, credible interval; CMCT, Central motor conduction time; Delta FS, the progression rate in ALS Functional Rating Scale-Revised (ALSFRS-R); FDI, first dorsal interosseous muscle; IQR, inter-quartile range; iSP, ipsilateral silent period; LMND, lower motor neuron dominant variants (including progressive muscle atrophy, flail arm, flail leg); N, sample size; SD, standard deviation; UMND, upper motor neuron dominant variants (including primary lateral sclerosis).

## 4. Discussion

We assessed transcallosal inhibition with the iSP protocol in a relatively large sample of ALS patients. In accordance with previous publications, we observed iSP loss in 54 % of the patients in our sample [7,9]. Furthermore, we were also able to demonstrate a structural neuroimaging correlate of this functional impairment, owing to a different methodological approach than in previous studies. The average fwFA values within the callosal tract between the primary motor cortices were lower for patients with iSP loss compared to patients with an iSP response. This group difference had a small effect size, and its detection seemed to require a larger sample size than was available in previous studies [7,9], as suggested by the sequential analysis. Therefore, the small effect size demonstrated by our hypothesis-directed analyses is not in opposition to the negative findings of previous studies.

Even though fwFA is minimally affected in the motor section of the corpus callosum in patients with iSP loss, other WM integrity metrics did not differ between patients with intact iSP and iSP loss, and a voxel-level group comparison within this callosal tract between the primary motor cortices did not identify any clusters. Regardless of this, a large proportion of ALS patients shows a marked disruption of the transcallosal inhibitory reaction. High rates of both increased latency of the iSP response and its complete loss have been described in ALS samples compared to control participants [7–9,11]. Thus, it would seem that even slight structural changes, which may not always be successfully identifiable with standard imaging analyses, may suffice to disrupt the normal transcallosal inhibition response. As also demonstrated by the previous studies, the functional TMS measurement is clearly a more sensitive index of transcallosal inhibition changes than structural metrics.

We further compared the patients with intact and deficient transcallosal inhibition regarding numerous demographic, clinical, and neuropsychological variables. We found an association with the CMCT, which was prolonged in ALS patients with iSP loss. Slowed corticospinal conduction has been commonly reported in ALS [37] and related to changes in WM metrics [38]. Besides this, we found anecdotal to strong evidence for the absence of a group effect for all other assessed variables, implying that the impairment of transcallosal inhibition is a specific characteristic in ALS patients that offers information distinct from demographic, clinical, and neuropsychological data. The motor section of the corpus callosum has been highlighted as one of the most markedly involved regions in ALS patients in diffusion imaging as well as post-mortem pathologic studies [5,39]. However, the reasons for the individual differences in the extent of WM structural changes in ALS patients and the impaired transcallosal inhibition that occurs in some of them need further investigation.

Some limitations of this study need to be considered. First, we chose to focus on the clear-cut dichotomous categorisation of transcallosal inhibition impairment to ensure an expectation of a high pathological load in the iSP loss group, which would be vital for discovering any possible co-occurrences with white matter structural changes. This choice of excluding the investigation of iSP latency values could have resulted in reduced sensitivity in identifying changes to the transcallosal inhibition. It should, however, also be noted, that the implications of increased iSP latency remain somewhat unclear. Second, whereas the iSP was measured from the FDI muscle at the start of data collection, the target was changed halfway through to the APB muscle, which has been shown to offer a more valid measurement [40]. Accordingly, a larger proportion of ALS patients in our sample showed a loss of iSP when measuring from the APB muscle (64 %) compared to the FDI muscle (46 %). Therefore, the overall prevalence of iSP loss may remain underestimated based on our data. Third, the presence of mirror movements as a clinical correlate of transcallosal inhibition impairment was not explored in our sample.

A valid measurement of iSP is only possible with sufficient innervation of the target muscle, which is a potential problem in ALS patients with advanced lower motor neuron degeneration. Indeed, in 6 % of our sample, measurement was only possible on one side due to the extent of lower motor neuron damage. Thus, future investigations should include compound muscle action potential amplitudes and muscle strength estimations to quantify the lower motor neuron degeneration and its influence on iSP measurement. Additionally, future studies could compare ALS to other neurodegenerative diseases in which patients display mirror movements and disrupted transcallosal inhibition such as asymmetric parkinsonian disorders as in early Parkinson's disease or corticobasal syndrome.

## 5. Conclusion

ALS patients with impaired transcallosal inhibition as assessed by the iSP showed reduced integrity of WM connections between the motor cortices. We identified no further phenotypical correlates of iSP loss, despite the relatively large sample size and the examination of numerous demographic, clinical, and neuropsychological parameters. Therefore, the reasons underlying these structural and functional changes in more

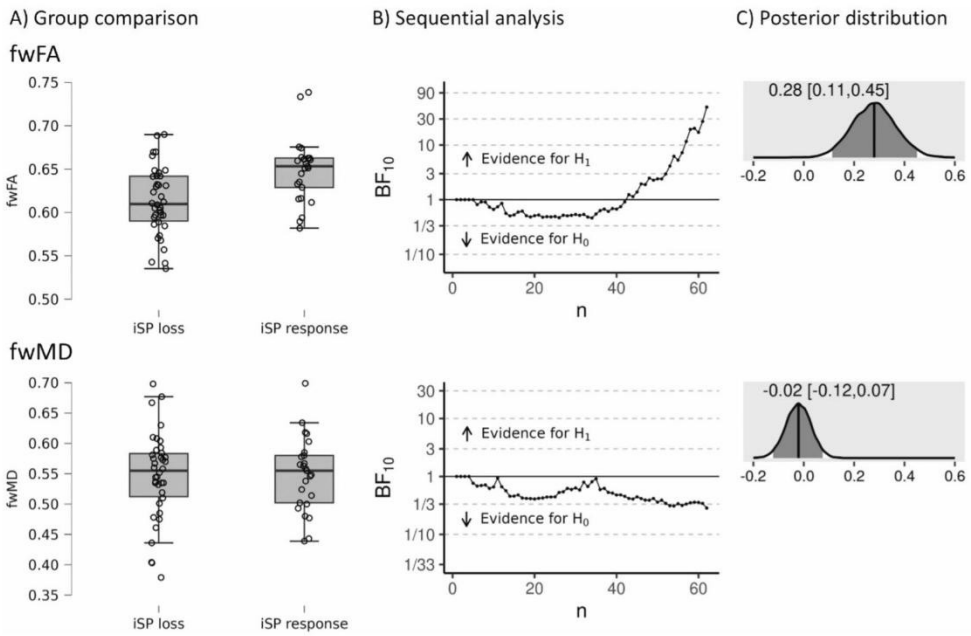


**Fig. 2.** Comparison of diffusivity metrics within the WM tract connecting primary motor cortices in patients with and without a loss of iSP. A) Box plots of fwFA values and fwMD values for the groups. In each box plot, the central line corresponds to the median, the upper and lower borders of the box to the 25th and 75th percentiles, respectively, and the length of the whiskers to 1.5 times the interquartile range. The fwMD values have been multiplied by 1000. B) Sequential analyses demonstrating the internal coherence of Bayesian ANCOVA for fwFA and fwMD data. The alternative hypothesis that the groups differ on the diffusivity metric reached a level of strong support ($BF_{10} > 10$) with the sequential inclusion of 58 cases for fwFA data. After the inclusion of all cases, there was very strong evidence for group differences on the fwFA metric and moderate evidence for a lack of group differences on fwMD metric. C) The posterior distributions of standardised parameter estimates for the effect of group for fwFA and fwMD data by Bayesian ANCOVAs. The median is marked with a solid line, the 95 % credible intervals are marked in grey, and the values are stated in each plot.

**Table 2**
The free-water-corrected diffusivity metrics and group differences.

| Diffusivity metric | iSP response group mean (SD) | iSP loss group mean (SD) | $BF_{10}$ |
|---|---|---|---|
| fwFA | 0.649 (0.037) | 0.613 (0.039) | 53.741 |
| fwMD | 0.549 (0.061) | 0.546 (0.072) | 0.281 |
| fwRD | 0.329 (0.045) | 0.346 (0.052) | 1.107 |
| fwAD | 1.015 (0.112) | 0.970 (0.129) | 0.498 |
| FWf | 0.220 (0.043) | 0.222 (0.043) | 0.481 |

$BF_{10}$, Bayes factor for the effect of group in Bayesian ANCOVA including age, sex, and metric value in control mask as covariates; fwAD, free-water-corrected axial diffusivity; FWf, free-water fraction; fwFA, free-water-corrected fractional anisotropy; fwMD, free-water-corrected mean diffusivity; fwRD, free-water-corrected radial diffusivity; iSP, ipsilateral silent period; SD, standard deviation. The values for fwMD, fwRD and fwAD have been multiplied by 1000.

than half of all ALS patients remain to be satisfactorily investigated, and further research (genetic investigations, in particular) would be beneficial.

## Ethical statements

The study was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients gave their written informed consent, and the study was approved by the local medical ethics committee.

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## CRediT authorship contribution statement

**Annaliis Lehto:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Julia Schumacher:** Writing – review & editing, Methodology. **Elisabeth Kasper:** Writing – review & editing, Conceptualization. **Stefan Teipel:** Writing – review & editing. **Andreas Hermann:** Writing – review & editing, Funding acquisition, Conceptualization. **Johannes Prudlo:** Writing – review & editing, Funding acquisition, Conceptualization.

## Declaration of competing interest

S.T. served on advisory boards of Lilly, Eisai, and Biogen, and is member of the independent data safety and monitoring board of the ENVISION study (Biogen).

## Data availability

The data analysed in the current study can be received from the corresponding author upon reasonable request from qualified investigators.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2024.123267.

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**Supplementary material**

| | N | iSP response | iSP loss | $BF_{10}$ | Mean (95% CI) |
|---|---|---|---|---|---|
| MoCA score | 91 | 24.0 (4.2) | 24.8 (3.6) | 0.313¤ | 0.082 (-0.107, 0.278) |
| ECAS total score | 88 | 103.6 (14.4) | 98.3 (15.8) | 0.356¤ | -0.094 (-0.287, 0.097) |
| ECAS language | 88 | 26 (3.0) | 25 (3.0) | 0.350¤ | -0.093 (-0.298, 0.108) |
| ECAS executive functions | 88 | 36.3 (6.2) | 35.6 (7.1) | 0.235¤ | 0.006 (-0.188, 0.203) |
| ECAS verbal fluency | 88 | 14.0 (4.1) | 12.3 (4.4) | 0.402¤ | -0.100 (-0.292, 0.090) |
| ECAS memory | 88 | 15.0 (4.6) | 13.9 (5.3) | 0.259¤ | -0.049 (-0.251, 0.150) |
| FrSBe total score | 27 | 53.0 (9.2) | 60.5 (17.4) | 0.673 | 0.187 (-0.161, 0.551) |
| FrSBe apathy | 27 | 57.0 (18.4) | 64.3 (13.9) | 0.602 | 0.167 (-0.171, 0.534) |
| FrSBe disinhibition | 27 | 43.9 (13.2) | 55.1 (13.5) | 1.787 | 0.310 (-0.043, 0.679) |
| FrSBe executive dysfunction | 27 | 48.2 (14.2) | 56.9 (14.4) | 0.862 | 0.222 (-0.127, 0.596) |

**Table S1** The cognitive and behavioural characterisation of the sample

$BF_{10}$, Bayes factor for the presence of group difference; CI, credible interval; ECAS, Edinburgh Cognitive and Behavioural ALS Screen; FrSBe, Frontal System Behaviour Scale; iSP, ipsilateral silent period; MoCA, Montreal Cognitive Assessment; N, sample size; ¤ Years of education included as a covariate

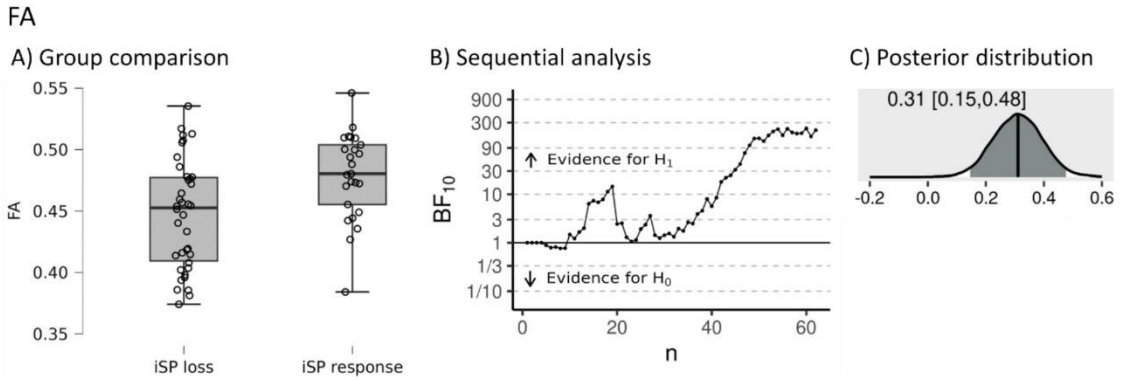


**Figure S1** Comparison of fractional anisotropy (FA) values within the tract connecting the primary motor cortices (without free water correction) in patients with and without a loss of iSP. A) Box plots of FA values for the groups. B) Sequential analysis demonstrating the internal coherence of Bayesian ANCOVA for FA data. With limited sample sizes, the support for the alternative hypothesis of group difference on FA is starkly affected by additions of single cases. A stable level of strong support ($BF_{10}$ > 10) is attained with the sequential inclusion of 43 cases, and with the inclusion of all cases, there is extreme evidence for a group difference. C) Posterior distribution of the standardised parameter estimate for the effect of group by Bayesian ANCOVA. The median is marked with a solid line, the 95% credible interval is marked in grey, and the values are stated in each plot.

| Diffusivity metric | iSP response group mean (SD) | iSP loss group mean (SD) | $BF_{10}$ |
|---|---|---|---|
| Fractional anisotropy | 0.479 (0.035) | 0.447 (0.044) | 256.793 |
| Mean diffusivity | 0.794 (0.094) | 0.800 (0.099) | 0.960 |
| Axial diffusivity | 1.218 (0.121) | 1.190 (0.133) | 0.418 |
| Radial diffusivity | 0.582 (0.087) | 0.606 (0.091) | 2.217 |

**Table S2** The non-free-water-corrected diffusivity metrics and group differences
Per diffusivity metric, the mean and standard deviation as well as the Bayes factor (from the standard diffusion model without free water correction) for the effect of group in Bayesian ANCOVA including age, sex, and metric value in control mask as covariates. The values for mean, axial, and radial diffusivity have been multiplied by 1000.

## 7.3. Inconsistent primary motor cortex glucose hypometabolism in primary lateral sclerosis

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ORIGINAL COMMUNICATION

# Inconsistent primary motor cortex glucose hypometabolism in primary lateral sclerosis

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## Abstract

**Objective** Primary lateral sclerosis (PLS) is a rare neurodegenerative disorder and a model for upper motor neuron degeneration, which is believed to begin in the primary motor cortex. However, clinical observation suggests that not all PLS cases show primary motor cortex glucose hypometabolism on 2-deoxy-2-[$^{18}$F]fluoro-D-glucose positron emission tomography (FDG-PET). We aimed to assess the reliability of FDG-PET in identifying motor cortex hypometabolism over disease course in a sample of patients with PLS.

**Methods** Baseline FDG-PET data from nine consecutive PLS patients were analyzed. At least one follow up assessment was available for five patients. We extracted the average FDG-PET signal in the primary motor cortex and other motor regions and calculated the covariate-corrected $z$ scores based on data of healthy controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.

**Results** Among the nine patients evaluated, only four demonstrated glucose hypometabolism in the primary motor cortex across available baseline and follow up assessments. Glucose metabolism of motor regions declined over time in some patients, whereas others maintained stable levels despite a worsening in symptom severity.

**Interpretation** Primary motor cortex hypometabolism in PLS patients is less consistent than previously reported, and the absence of this hypometabolic sign should not be considered as irrefutable evidence against PLS in the diagnostic process. The findings of our study underline the heterogeneity of PLS, indicating that more precise diagnostic tools would be beneficial to confirm a PLS diagnosis at an earlier stage.

**Keywords** Primary lateral sclerosis · Cerebral glucose metabolism · FDG-PET · Primary motor cortex

## Introduction

Primary lateral sclerosis (PLS) is a rare neurodegenerative bulbospinal upper motor neuron syndrome [1, 2]. Although most cases of clinical PLS have cortical ubiquitin and TAR DNA-binding protein 43 (TDP-43) pathology [3, 4] and the PLS phenotype may appear as part of the phenotypic spectrum in familiar ALS pedigrees [5], PLS is no more considered part of the ALS spectrum by the Gold Coast Criteria

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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[6]. The reasons for this are the lack of lower motor neuron signs and a significantly slower rate of progression when compared with classic ALS. Mills' syndrome is a unilateral upper motor neuron disease, which appears clinically in the form of a slow progressive spastic hemiparesis; it is a rare variant of PLS [7, 8]. Despite the upper motor neuron impairment being the defining characteristic of PLS, radiological evidence suggests that PLS patients may also show widespread grey or white matter alterations beyond the cortical regions of motor control and the corticospinal tracts [9, 10]. In contrast to classic ALS, diagnosing PLS is challenging, especially in the first two years of the disease, as it often requires diverse and targeted investigations to exclude a multitude of other diseases (e.g. inflammatory central nervous system disorders, compressive myelopathy) and to distinguish it from mimics such as upper motor neuron-dominant ALS, hereditary spastic paraplegia (HSP), and progressive supranuclear palsy (PSP) [11–13]. Sensitive diagnostic biomarkers of PLS are still lacking and further diagnostic tools would be valuable.

PLS is a disease model for upper motor neuron degeneration, apparently beginning in the primary motor cortex (M1), which is why glucose hypometabolism in the precentral cortex would be expected to be detectable on a 2-deoxy-2-[$^{18}$F]fluoro-D-glucose-positron-emission tomography (FDG-PET) scan. Indeed, some case reports support this notion [14–17], encouraging the utilization of an FDG-PET assessment in the differential diagnosis. However, clinical observations have revealed this not always to be the case, which prompted this study to investigate M1 hypometabolism in a sample of nine PLS cases both cross-sectionally and longitudinally. The main aim of the current study was to examine the reliability of FDG-PET in identifying M1 hypometablism in a consecutive sample of patients who fit the diagnostic criteria for PLS. We broadened our scope to include further motor regions, motivated by previous reports of structural changes in these wider areas [18, 19].

## Methods

### Participants

Our sample consisted of nine consecutive patients recruited via the Department of Neurology of the Rostock University Medical Centre who met the consensus diagnostic criteria for probable or definite PLS [1]. All patients completed at least one clinical and FDG-PET imaging assessment. Furthermore, all patients underwent a routine magnetic resonance imaging (MRI) with fluid-attenuated inversion recovery, T1-weighted and T2-weighted protocols to rule out alternative pathologies. Six of the nine patients went through genetic testing of the common ALS/PLS-causing genes linked to ALS. One out of the nine had an ALS/PLS-causing mutation, five were screened negative, and no genetic data was available for three cases. The ALS Functional Rating Scale Revised (ALSFRS-r) was administered and subdomain scores were calculated (items 1–3 for bulbar score, items 4–6 for upper limb score, items 8 and 9 for lower limb score).

Furthermore, the baseline FDG-PET images of 70 healthy controls (HCs) from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu) as described in a previous study [20] were included in the analysis. The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment and early Alzheimer's disease. For up-to-date information, see www.adni-info.org.

### FDG-PET acquisition and preprocessing

FDG-PET data of HC were obtained in preprocessed form from the ADNI database. These images were acquired using various scanners, following platform-specific protocols. To ensure consistency across different scanners, all original ADNI FDG-PET scans underwent standardized preprocessing steps. Comprehensive details about the acquisition and preprocessing of FDG-PET images can be found on the ADNI website (https://adni.loni.usc.edu/data-samples/adni-data/neuroimaging/pet/).

The PLS patients underwent dynamic PET imaging (4 × 5 min) of the brain using a Gemini TF 16 scanner (Philips Healthcare) at 30 min after the injection of 199 ± 18 MBq FDG. Prior to PET imaging, an auxiliary CT scan (120 kVp, 30 mAs) was performed. Four dynamic PET frames were recorded, examined for head movements and any conspicuous frames were excluded. A static PET data set was reconstructed using the manufacturer's proprietary BLOB-OS reconstruction algorithm (3 iterations, 31 subsets), which was corrected for randoms, scatter, decay, and attenuation using information from the auxiliary CT. To match the spatial resolution of 6 mm of the ADNI data, the FDG-PET scans of the PLS patients were filtered to the identical resolution using a Butterworth filter. All FDG-PET scans were skull-stripped, intensity normalized using the scan average, and registered to an FDG-PET template [21]. No partial volume correction was applied to the FDG-PET scans.

### Regions of interest (ROIs)

For all participants, ROI masks were used to extract the average regional FDG-PET signal. The ROIs included: the primary motor cortex (M1), dorsal and ventral premotor cortex (PMd, PMv), supplementary motor area (SMA), and pre-supplementary motor area (pre-SMA) from the Human Motor Area Template [22] and the upper limb, lower limb, trunk, head and face, tongue and larynx regions from the Brainnetome Atlas [23].

### Statistical analysis

The statistical analyses were completed and figures were created in R (https://www.r-project.org/). To determine whether the PLS patients had extreme values of FDG uptake in investigated ROIs, the mean signal intensity of each ROI was expressed as a w-score, which represents a covariate-adjusted z score derived from a control group distribution. The procedure involved two main steps. First, the FDG signal for each ROI in the HCs was regressed against age and sex, yielding regression coefficients and residuals. These regression coefficients were subsequently applied to calculate residual values for the PLS patients. Second, the mean and standard deviation (SD) of the residuals in the HC group were utilized to calculate the respective *w* scores for the patient residuals. This adjustment enabled the identification of disease-related alterations in FDG uptake while controlling for age and sex differences. *W* scores follow the *z* distribution and are therefore directly translatable to *p* values. A *w* score $< -1.65$ indicates a one-tailed *p* value below 0.05 and a *w* score $< -1.96$ indicates a one-tailed *p* value below 0.025. For a longitudinal assessment, *w* scores were also determined for available follow up (FU) visits. *W* scores of all assessed regions for all patients can be found in Supplementary Table 1.

## Results

### Demographic and clinical data

A multifaceted characterization of our sample can be found in Table 1. The patients ($N = 9$) varied greatly in their age (mean $= 61 \pm 8$ years) and disease duration at baseline (mean $= 40 \pm 24$ months). Five patients (44%) displayed pseudobulbar affect (PBA). 44% of the patients were female when compared with 54% of the HCs. The HCs were on average older than the PLS patients (mean $= 72 \pm 5$ years).

Two patients (pat3 and pat9) initially showed slowly progressive unilateral upper motor neuron signs, corresponding to Mills' syndrome [7, 16, 24, 25]. The symptoms started in the left lower limb for patient 3 and the right lower limb for patient 6. Over a period of 3 years, patient 3's clinical signs spread from the left leg to the left hand, then to the right leg, right hand, and lastly to the bulbar region. Patient 6's clinical signs spread over the course of 5 years from the right leg to the left leg, then the right hand and the left hand. Although both patients became bilaterally affected, the impairments remained asymmetrically more severe on the onset side of the body. Neither patient 3 nor patient 6 showed lower motor neuron signs on the clinical or electrodiagnostic assessments. Patient 3 showed asymmetric frontotemporal atrophy in the right hemisphere, whereas patient 6 showed light nonspecific atrophy. The cerebrospinal fluid examination and further investigations did not reveal other pathologies. The genetic analysis was negative for pat6, but a mutation in the TPK1-gene of uncertain significance was discovered in patient 3.

At the baseline assessment, six patients of our sample already had a symptomatic involvement of all three regions (bulbar, upper limb, and lower limb). For all the following results, right and left pertain to respective hemispheres.

### FDG-PET data

Despite the considerable symptomatic burden, only four of the patients showed regional hypometabolism (w scores $< -1.96$) in motor regions (whole M1 or a subregion of M1): patients 3, 4 and 6 from baseline onwards and patient 1 only from the first FU onwards (Table 1). The patients' regional *w* scores have been projected onto the standard normal distributions in Fig. 1.

Despite the bulbar, upper limb, and lower limb involvement, all regional values of patient 1 stayed above $w = -2$ at the baseline assessment. At FU assessments, patient 1 showed a bilateral decrease in the FDG uptake in M1 (right hemisphere FU1 $w = -3.2$ to FU2 $w = -3.2$, and left hemisphere FU1 $w = -3.3$ to FU2 $w = -3.5$). Patient 1 also had low *w* scores for the head and face region of M1 (right FU1 $w = -3.3$ to FU2 $w = -3.6$, and left FU1 $w = -3.0$ to FU2 $w = -3.8$) and for the upper limb region (right FU2 $w = -2.6$, and left FU1 $w = -2.2$ to FU2 $w = -2.6$). Furthermore, patient 1 showed low FDG uptake in the left SMA (FU1 $w = -2.3$ to FU2 $w = -2.9$), the left pre-SMA (FU2 $w = -2.1$), and the PMv (right FU1 $w = -3.4$ to FU2 $w = -4.2$, and left FU1 $w = -2.9$ to FU2 $w = -4.2$).

Patient 3 had asymmetric lower and upper limb involvement at baseline (Mills' syndrome), and at FU1 the bulbar region had also become symptomatic. At baseline, patient 3 showed a decrease in the FDG uptake in the right M1 ($w = -2.3$). At FU1, the *w* scores were low for the bilateral M1 (right $w = -3.2$ and left $w = -2.6$), the right upper limb region ($w = -3.3$), the right SMA ($w = -3.1$), and the right PMd ($w = -2.4$).

**Table 1** Demographic and clinical characteristics of the PLS patients

| Patients | Age at B | Sex | Probable vs definite PLS | Disease duration at B (months) | Onset region | Symptomatic regions at BL | Bulbar score (max 12) | Upper limb score (max 12) | Lower limb score (max 8) | ALSFRS-r total (max 48) | PBA | Genetics | Whole M1 hypomet. at BL | BL to FU1 (months) | Whole M1 hypo-met. at FU1 | FU1 to FU2 (months) | Whole M1 hypo-met. at FU2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pat1 | 47 | Female | Definite | 54 | Pseudobulbar | BUL, UL, LL | 7 | 5 | 3 | 28 | Yes | Negative | No | 47 | Bilateral | 24 | Bilateral |
| Pat2 | 76 | Female | Definite | 14 | Pseudobulbar | BUL | | | | 42 | Yes | Negative | No | 12 | No | 22 | No |
| Pat3 | 63 | Male | Probable | 7 | Left lower limb | LL, UL | | | | 40 | No | TBK1 | Right hemisphere | 17 | Bilateral | | |
| Pat4 | 62 | Female | Definite | 21 | Pseudobulbar | BUL, UL, LL | 8 | 8 | 3 | 34 | Yes | Not tested | Left hemisphere | 11 | Left hemisphere | | |
| Pat5 | 58 | Female | Probable | 26 | Left lower limb | LL, UL, BUL | 8 | 8 | 3 | 33 | No | Negative | No | 15 | No | | |
| Pat6 | 66 | Male | Definite | 39 | Right lower limb | LL | | | | 35 | No | Negative | No | | | | |
| Pat7 | 55 | Male | Definite | 56 | Pseudobulbar | BUL, UL, LL | 8 | 10 | 8 | 41 | No | Not tested | No | | | | |
| Pat8 | 65 | Male | Definite | 65 | Bilateral lower limb | LL, UL, BUL | 8 | 4 | 2 | 25 | Yes | Negative | No | | | | |
| Pat9 | 58 | Male | Definite | 74 | Left upper limb | LL, UL, BUL | | | | 31 | Yes | Not tested | No | | | | |

Probable vs definite PLS pertains to the last available follow up of the patient and not to the classification at baseline. Bulbar score is the sum of items 1–3, the upper limb score is the sum of items 4–6 and lower limb score is the sum of items 8–9 of the ALSFRS-r. A *w* value below − 1.96 indicated hypometabolism. More information on *w* scores and their corresponding p values can be found in the methods section

*ALSFRS-r* ALS Functional Rating Scale Revised, *BL* baseline, *BUL* bulbar, *FU* follow up, *hypomet* hypometabolism, *L* left, *LL* lower limb, *M1* primary motor cortex, *R* right, *PBA* pseudobulbar affect, *TBK1* mutation in the TANK-binding kinase 1 gene, *UL* upper limb

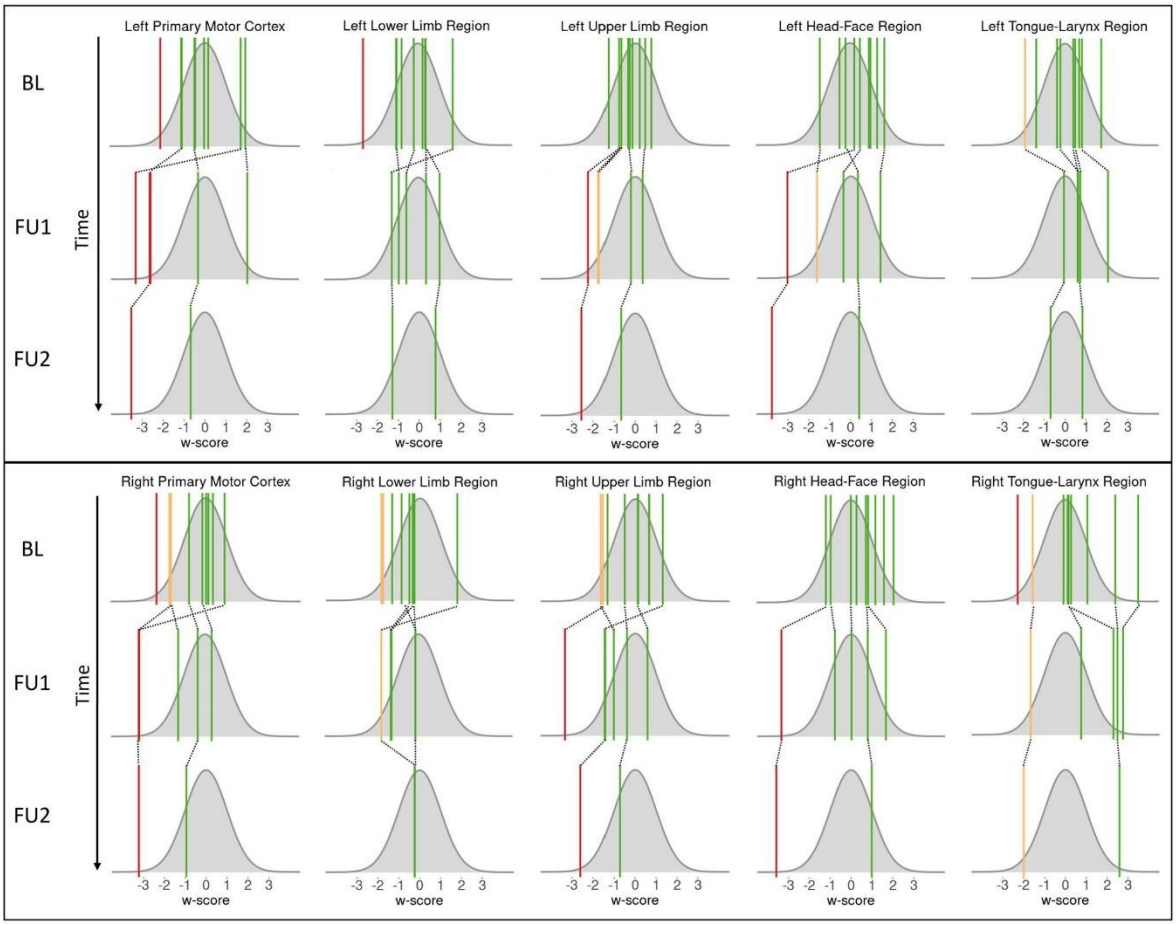


**Fig. 1** Regional *w* scores of PLS patients for available time points. The *w* scores of PLS patients are visualized with colored lines over a normal distribution. *W* scores > − 1.65 are shown in green (one-sided *p* value > 0.05), *w* scores between − 1.65 and − 1.96 are shown in yellow (one-sided *p* values between 0.05 and 0.025), and *w* scores < − 1.96 are shown in red (one-sided *p* value < 0.025). Right and left refer to the assessed hemisphere. *BL* baseline, *FU* follow up

At baseline, patient 4 had symptoms in the bulbar region, lower, and upper limbs, and the FDG uptake was low in the left M1 at both baseline ($w = -2.1$) and at FU1 ($w = -2.6$).

The baseline scan of patient 6 with lower limb involvement showed low *w* scores for the left lower limb region ($w = -2.6$) and for the right tongue and larynx region ($w = -2.3$). Furthermore, patient 6 had low FDG uptake in the bilateral SMA (right $w = -2.1$ and left $w = -3.4$), and left PMd ($w = -2.6$).

The regional scores of the remaining five patients were all > − 1.96. *W* scores of all assessed regions for all patients can be found in Supplementary Table 1.

## Discussion

We aimed to assess the reliability of FDG-PET in identifying UMN degeneration in a consecutive sample of PLS patients. Among the nine patients evaluated, only four demonstrated glucose hypometabolism in the primary motor cortex across available baseline and follow up assessments. Of those four patients, three also exhibited glucose hypometabolism in motor regions beyond the primary motor cortex. Glucose metabolism of motor regions declined over time in some patients, whereas others maintained stable metabolic levels despite

worsening symptom severity. No consistent relationships were observed between glucose metabolism levels and clinical variables such as age, disease duration, or symptom distribution. These findings suggest that the primary motor cortex hypometabolism in PLS patients is less consistent than previous case reports have indicated [14–16]. Consequently, the absence of this hypometabolic sign should not be considered as irrefutable evidence against a diagnosis of PLS.

Glucose metabolism is closely linked to neural activity and the observed interindividual variability in our findings may have several possible explanations. One reason could be that the cortical changes in the M1 are not conspicuous in all patients that meet the PLS diagnostic criteria. Although a loss of Betz cells in the primary motor cortex is reported in many autopsy studies [4], multiple PLS cases have also revealed corticospinal tract degeneration without a discernible loss of Betz cells [26]. MRI studies have revealed precentral gyrus atrophy in PLS when compared with healthy controls on the group level [27–29] but also clearly highlighted its progressive nature over the disease course for individual patients [18, 30]. FDG-PET may not be sensitive enough to identify limited neurodegeneration in the first stages of the disease in each PLS patient.

Further between-patient variability in glucose metabolism in our sample may be related to differences in the underlying pathology. Because no autopsy data are available for the patients in our sample, the pathologies leading to the clinical phenotypes meeting the PLS criteria cannot be determined. A renowned autopsy report on patients with PLS phenotype describes a degeneration of the M1 and corticospinal tracts, numerous TDP-43 inclusions in the M1, limited or no inclusions in the lower motor neurons and further inclusions in extramotor neocortex [3, 4]. Nevertheless, also other pathologies, such as tau can lead to the clinical phenotype of PLS [12, 31]. A recent clinico-pathological case series of patients that filled the PLS diagnostic criteria highlighted both the pathological variability that may lead to this phenotype and the difficulties of differential diagnosis [32].

One further reason for the variability in our sample could be a heterogeneous spread of PLS pathology: it may be that the pathological spread is not only anterograde or "dying forward" (whereby degeneration spreads from the soma of the Betz cell to the distal axonal parts of the upper motor neuron) [33, 34] but also retrograde or "dying back" (in which degeneration spreads from the distal axonal parts of the upper motor neuron to the soma of the Betz cell) [35]. For instance, about 50% of PLS patients exhibit a symmetric ascending pattern of paralysis (from the lower limbs to the upper limbs to the bulbar regions), which supports the hypothesis of length-dependent dying back of corticospinal axons, whereas other patients show a more asymmetric or "patchy" spread of symptoms [36].

Finally, the precise cellular source of the FDG-PET signal remains undefined. Beyond neuronal glucose consumption, FDG uptake in cerebral tissue is dependent on astrocytes [37] and microglia [38]. A comprehensive overview of the relative contributions of these cell types to the FDG signal has yet to be provided. Previous PET studies in PLS have shown that both neuronal death [39] and glial activation, which co-localizes with grey matter atrophy [40, 41], exert opposing effects on the FDG-PET signal. Therefore, further investigations are required to clarify the cellular and molecular mechanisms underlying FDG signal changes in individual patients, providing a deeper understanding of disease pathology and its heterogeneity.

The diagnosis of PLS remains challenging, particularly its differentiation from UMN-dominant ALS, PSP and HSP [11, 13]. So far, no specific diagnostic biomarkers exist to enhance the diagnostic accuracy of PLS, necessitating a broad array of assessments including magnetic resonance imaging and transcranial magnetic stimulation [13, 32]. FDG-PET has been proposed as a potentially sensitive diagnostic tool; however, our findings suggest that its utility in clinical practice for diagnosing PLS may be limited and unreliable.

Our study is not without limitations. Like many other PLS studies, our sample size was small due to the rarity of this disease. Furthermore, in our sample, the HCs were older than the patients. Previous publications have reported primary motor cortex FDG-PET signal to remain relatively unaffected by age-related change [42] and found only a moderate decrease over the span of 40–85 years [43]. To minimize the possible age effect on the FDG-PET signal in our sample, we utilized covariate-adjusted $w$ scores. In addition, the subregions of the primary motor cortex included in our analysis were relatively small and the spatial resolution of the imaging data (6 mm) introduced potential partial volume effects. Such effects may have led to spill-in signals from neighboring regions, potentially confounding the true FDG uptake in smaller areas and hindering the detection of localized metabolic changes. To identify subtle, small-scale changes, PET cameras with higher spatial resolution should be considered in future investigations.

In conclusion, FDG-PET does not appear to be a reliable diagnostic tool for PLS, particularly in the early disease stages when a sensitive and reliable biomarker would be most beneficial. Therefore, caution should be exercised when using FDG-PET as an additional diagnostic tool in suspected cases of PLS. Despite the challenges, there is an increasing interest in the delineation of the clinical entity of PLS. The findings of our study underline the heterogeneity within the patients that meet the diagnostic criteria of PLS. Future studies with larger cohorts are needed to validate the present findings and to investigate the clinical and biological features underlying this individual variability in PLS.

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**Author contributions** Annaliis Lehto: conceptualization, formal analysis, methodology, visualisation, writing—original draft, writing—review and editing. Julia Schumacher: methodology, writing—review and editing. Jens Kurth: writing—review and editing. Bernd Joachim Krause: funding acquisition, writing—review and editing. Elisabeth Kasper: conceptualization, writing—review and editing. Stefan Teipel: conceptualization, writing—review and editing. Johannes Prudlo: conceptualization, funding acquisition, writing—review and editing.

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**Data Availability** The data analyzed in the current study can be received from the corresponding author upon reasonable request from qualified investigators.

## Declarations

**Conflicts of interest** S.T. served on advisory boards of Lilly, Eisai, and Biogen, and is member of the independent data safety and monitoring board of the ENVISION study (Biogen).

**Ethics declarations** The study was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients gave their written informed consent, and the study was approved by the local medical ethics committee.



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**Inconsistent primary motor cortex glucose hypometabolism in primary lateral sclerosis**

Journal of Neurology

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| | Time | Duration | Affected regions | M1_R | M1_L | hf_L | hf_R | ul_L | ul_R | tl_L | tl_R | ll_R | ll_L | SMA_R | SMA_L | preSMA_R | preSMA_L | PMd_R | PMd_L | PMv_R | PMv_L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pat1 | 1 | 54 | BUL, UL, LL | 0.90 | 1.70 | 0.20 | -1.19 | 0.76 | 1.31 | -1.92 | -1.55 | 1.78 | 1.66 | 2.29 | 0.91 | 0.78 | 0.67 | 1.83 | 2.73 | -1.74 | -1.29 |
| | 2 | 101 | BUL, UL, LL | -3.16 | -3.32 | -3.04 | -3.34 | -2.24 | -1.44 | -0.06 | -1.67 | -1.78 | -1.27 | -1.93 | -2.25 | -0.35 | -0.43 | -1.05 | -0.24 | -3.40 | -2.95 |
| | 3 | 125 | BUL, UL, LL | -3.22 | -3.52 | -3.77 | -3.58 | -2.55 | -2.60 | -0.69 | -2.00 | -0.24 | -1.28 | -1.39 | -2.95 | -1.54 | -2.13 | -1.38 | -1.94 | -4.21 | -4.20 |
| pat2 | 1 | 14 | BUL | -0.78 | -0.45 | -0.22 | 0.72 | -0.27 | -0.51 | -0.22 | 2.42 | -0.35 | 0.34 | 0.29 | 0.89 | 0.49 | 0.31 | 0.82 | 0.01 | 2.99 | -0.05 |
| | 2 | 26 | BUL | -0.35 | -0.33 | 0.33 | 0.80 | -0.21 | -0.39 | 0.63 | 2.49 | -0.16 | 1.02 | 0.74 | 1.37 | -0.24 | -0.15 | 0.65 | 0.08 | 2.40 | -0.45 |
| | 3 | 48 | BUL, UL | -0.94 | -0.69 | 0.41 | 0.99 | -0.67 | -0.73 | 0.83 | 2.59 | -0.24 | 0.78 | 0.52 | 1.28 | 0.38 | 0.59 | 0.55 | 0.19 | 2.60 | -0.01 |
| pat3 | 1 | 7 | LL, UL | -2.32 | -1.11 | 0.47 | 0.00 | -0.35 | -1.53 | 0.82 | 0.18 | -0.33 | -0.20 | -1.35 | -0.43 | -0.46 | -0.02 | -1.19 | 0.74 | 0.00 | 1.07 |
| | 2 | 24 | LL, UL, BUL | -3.17 | -2.65 | -0.36 | 0.02 | -1.71 | -3.33 | 2.05 | 2.30 | -1.29 | -0.57 | -3.14 | -1.54 | -1.00 | -0.50 | -2.40 | 0.18 | 0.36 | 1.56 |
| pat4 | 1 | 21 | BUL, UL, LL | -1.64 | -2.10 | -1.44 | -0.97 | -1.28 | -1.63 | 0.40 | 0.14 | -1.34 | -1.05 | -1.58 | -1.02 | -1.64 | -1.22 | -1.77 | -0.61 | -0.95 | -0.18 |
| | 2 | 32 | BUL, UL, LL | -1.28 | -2.60 | -1.63 | -0.78 | -1.76 | -1.03 | 0.60 | 0.76 | -1.33 | -0.93 | -1.25 | -0.93 | -1.18 | -0.75 | -1.30 | -1.12 | -0.75 | -1.23 |
| pat5 | 1 | 26 | LL, UL, BUL | -0.15 | 1.92 | 1.64 | 0.82 | 0.47 | 0.11 | 0.49 | 3.52 | -0.51 | 0.22 | 0.16 | 1.13 | 0.69 | 0.56 | 0.08 | 1.21 | 3.48 | 3.72 |
| | 2 | 41 | LL, UL, BUL | 0.32 | 2.02 | 1.41 | 1.67 | 0.35 | 0.59 | 0.72 | 2.76 | -0.15 | 0.37 | 0.85 | 1.50 | 1.29 | 1.30 | 0.34 | 1.74 | 4.20 | 3.48 |
| pat6 | 1 | 39 | LL | 0.35 | -1.09 | -0.50 | 2.04 | -0.78 | 0.13 | -1.39 | -2.26 | -1.85 | -2.63 | -2.12 | -3.39 | -0.33 | -1.01 | -0.73 | -2.55 | 0.78 | -1.14 |
| pat7 | 1 | 56 | BUL, UL, LL | 0.03 | 0.17 | 1.30 | 1.58 | -0.67 | 0.65 | 0.69 | 0.30 | -0.89 | -1.02 | -0.42 | -0.07 | -0.17 | 0.37 | 1.46 | 0.81 | 2.04 | 2.38 |
| pat8 | 1 | 65 | LL, UL, BUL | 0.13 | -0.49 | 0.98 | 0.27 | 0.21 | 0.67 | -0.38 | -0.07 | -1.76 | -0.79 | -0.95 | -0.02 | -0.35 | 0.54 | 1.64 | 2.65 | 2.19 | 2.06 |
| pat9 | 1 | 74 | LL, UL, BUL | -1.72 | -0.03 | 0.90 | 1.17 | -0.14 | -1.33 | 1.74 | 1.08 | -0.27 | 0.36 | -0.18 | 0.21 | 0.43 | 0.54 | -0.61 | -0.29 | 2.28 | 3.13 |

**Supplementary table 1.** Regional w-scores of PLS patients

Duration is given in months and affected regions entail regions where patients report noticeable symptomatic involvement. W-scores between -1.65 and -1.96 are shown in light orange (one-sided p-values between 0.05 and 0.025), and w-scores < -1.96 are shown in dark orange (one-sided p-value < 0.025). Right and left refer to the assessed hemisphere. BUL, bulbar; hf, head and face; L, left; ll, lower limb; M1, primary motor cortex; PMd, dorsal pre-motor cortex; PMv, ventral pre-motor cortex; R, right; SMA, supplementary motor area; tl, throat and larynx; ul, upper limb.

# 8. Appendix

## 8.1. Acknowledgements

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## 8.2. Summary

The aim of this dissertation was to investigate brain structure and glucose metabolism changes in amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS) in order to more accurately describe their correlations with patients' motoric, cognitive or behavioural characteristics. The three studies analysed clinical, neuropsychological, imaging and neurophysiological data from well-characterised ALS and PLS patients by utilizing valuable multimodal datasets.

Key findings were as follows: the fronto-temporal glucose hypometabolism in ALS patients seems to correspond more to behavioural than cognitive impairments. Cognitive functioning in ALS patients in different domains as assessed with the ECAS instrument is not strongly related to regional glucose metabolism or grey matter volume changes. A transcallosal inhibition deficit is associated with a reduced integrity of white matter connections between the motor cortices. However, since transcallosal inhibition deficit neither corresponds with a specific phenotype of ALS nor with the rate of disease progression, it does not serve as a categorical or prognostic biomarker in ALS. Moreover, FDG-PET does not reliably identify primary motor cortex glucose hypometabolism in PLS and has thus only limited value in a differential diagnosis.

The findings from the studies presented here illuminate multiple aspects of heterogeneity among patients with ALS and PLS in addition to improving our understanding of the neuroimaging correlates of specific phenotypic characteristics in those patient groups.

## 8.3. Curriculum Vitae

### Personal details

| | |
|---|---|
| Name | Annaliis Lehto |
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| Date of birth | 14.07.1996 |

### Educational qualification

09/2019 – 08/2021 **Maastricht University** (Netherlands)
Research Master of Cognitive and Clinical Neuroscience: Specialisation Neuropsychology, cum laude (Grade: 8.57/10)
Master thesis: „The assessment of cortical inhibition and excitation in patients with akinetic-rigid subtype of Parkinson's disease" (Grade: 9.5/10)

09/2016 – 07/2019 **Maastricht University** (Netherlands)
Bachelor of Psychology and Neuroscience, cum laude (Grade: 8.8/10)
Bachelor thesis: „How do we learn rhythms? The roles of prediction, movement and individual differences in rhythm perception and learning" (Grade: 9/10)

09/2013 – 06/2015 **Regent's International School Bangkok** (Thailand)
International Baccalaureate Diploma Programme (Score: 37/45)

09/2002 – 06/2013 **Kuressaare Gümnaasium** (Estonia)
Primary and secondary school

### Professional qualification and research experience

03/2022 – 06/2025 **PhD student at the Clinic of Neurology, Rostock**
Supervised by Prof. Dr. med. Prudlo
Diverse analyses of clinical and imaging data in amyotrophic lateral sclerosis and primary lateral sclerosis to answer a wide range of research questions

11/2020 – 06/2021 **Research internship at the University Clinic Düsseldorf**
Supervised by Dr. med. Groiss und Prof. Dr. med. Wojtecki
Conducting a transcranial magnetic stimulation study on the differences in cortical excitability and inhibition in different tremor diseases

11/2020 – 06/2021 **Clinical internship at the Hospital zum Heiligen Geist, Kempen**
Supervised by Prof. Dr. med. Wojtecki
Establishment of a data registry and subsequent analyses on treatment effects of deep transcranial magnetic stimulation and transcranial pulse stimulation

10/2018 – 06/2019 **Research internship at the Maastricht University**
Supervised by Dr. Brown
Carrying out an EEG study on individual differences in rhythm perception

01/2018 – 03/2018 **Research assistant for Dr. Thomson, Maastricht University**
Assistance with a transcranial magnetic stimulation experiment

2017 – 2020 **Tutor for a variety of courses at the Maastricht University**
For instance: Statistics 1 & 2, Perception, Personality and Individual Differences.

## Publications

1. Lehto, A., Zapf, A., Hermann, A. et al. (2025). Homozygosity for the C allele at UNC13A rs12608932 seems to compromise cognition in ALS independently of the cognitive domains. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 1-4. https://doi.org/10.1080/21678421.2025.2608238
2. Lehto, A., Schumacher, J., Teipel, S. *et al.* Cerebellar grey matter volume is associated with semantic fluency performance in amyotrophic lateral sclerosis patients. *Brain Communications*, fcaf230. https://doi.org/10.1093/braincomms/fcaf230
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