Temporal lobe pathology in Amyotrophic Lateral Sclerosis

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Zusammenfassung

Die Amyotrophe Lateralsklerose (ALS) zählt zu den häufigsten Motoneuronerkrankungen des Erwachsenenalters und ist gekennzeichnet durch eine rasch fortschreitende Degeneration des ersten und zweiten Motoneurons der Pyramidenbahn. Die Erkrankung galt lange Zeit als rein motorische Erkrankung ohne Beteiligung anderer Funktionssysteme. Untersuchungen der letzten zwei Jahrzehnte haben jedoch demonstriert, dass es bei etwa der Hälfte der Patienten zu kognitiven Störungen und Veränderungen des Verhaltens kommen kann, von denen bis zu 15% die Kriterien für eine Frontotemporale Demenz (FTD) erfüllen. Hinweise für eine gemeinsame Pathologie mit FTD gibt es auch aus histopathologischen und molekulargenetischen Untersuchungen, die zeigen, dass bei beiden Erkrankungen pathologisch veränderte ubiquitin-positive Einschlüsse des Proteins TDP-43 in den Nervenzellen nachweisbar sind und es eine gemeinsame Mutation in einer Wiederholungssequenz des C9orf72 Gens auf Chromosom 9 gibt. Ergebnisse aus Bildgebungsstudien legen zudem nahe, dass bei der ALS neben Veränderungen im motorischen System auch extra-motorische Areale des Frontal- und Parietallappens betroffen sein können. Pathologische Veränderungen im Temporallappen wurden dagegen bislang eher selten berichtet. Das ist vor allem in Hinblick auf eine gemeinsame Pathologie mit den Frontotemporalen Demenzen von Relevanz, die mit degenerativen Veränderungen des Frontal- und Temporallappens einhergehen. Vor diesem Hintergrund war die Zielsetzung der vorliegenden Arbeit, inwiefern verhaltensrelevante, strukturelle und funktionelle pathologische Veränderungen des Temporallappens mit der ALS assoziiert sind.

In der ersten Studie wurde dazu das Gedächtnisprofil von ALS Patienten mit dem solcher Patienten verglichen, die an einer leichten kognitiven Beeinträchtigung im Gedächtnisbereich leiden (aMCI) und als Vorstufe der Alzheimererkrankung gelten. Die Ergebnisse zeigen, dass ALS Patienten Defizite im Gedächtnisbereich aufweisen, diese jedoch unterschiedlich von jenen Defiziten sind, die bei den aMCI Patienten zu beobachten sind. Die Wiedererkennungsleistung der ALS Patienten war signifikant reduziert, wohingegen sich der kurz- und langfristige Abruf verbalen Materials als unbeeinträchtigt darstellten. Das Profil der aMCI Patienten wies hingegen Defizite des kurz- und langfristigen Abrufs mit unbeeinträchtigter Wiedererkennungsleistung auf. Basierend auf diesen Ergebnissen untersuchte Studie 2, inwiefern das gezeigte Profil der ALS Patienten auch mit einem

strukturellen Defizit innerhalb des Temporallappens zu vereinbaren ist. Dazu wurden volumetrische und formrelevante Veränderungen des Hippokampus mittels struktureller Magnetresonanztomografie (MRT) untersucht. Der Hippokampus ist primär an der Verarbeitung und Speicherung neuer Gedächtnisinhalte beteiligt und daher von zentralem Interesse, wenn es um die anatomischen Korrelate von Gedächtnisdefiziten geht. Studie 2 konnte zeigen, dass der Hippokampus im Krankheitsverlauf in Form eines reduzierten Volumens sowie lokaler struktureller Veränderungen in der CA1-Region im Kopf des Hippokampus betroffen sein kann.

Während die ersten beiden Studien ausschließlich auf die Untersuchung von Veränderungen des Gedächtnisses ausgerichtet waren, wurden in der 3. Studie auch andere neuropsychologische Veränderungen der Patienten und deren strukturelle Korrelate in Betracht gezogen. Dazu wurde mittels neuropsychologischer Verfahren und struktureller MRT untersucht, ob subkortikale Kerngebiete des Temporallappen (oder mit Verbindungen zu Strukturen des Temporallappens) in Abhängigkeit von unterschiedlich stark ausgeprägten kognitiven und verhaltensrelevanten Defiziten im Rahmen der Erkrankung Veränderungen aufweisen. Dabei konnte gezeigt werden, dass, basierend auf den unterschiedlichen kognitiven Phänotypen der ALS, eine Volumenreduktion der untersuchten subkortikalen Strukturen mit dem Grad der Ausprägung der Defizite einhergeht. Dabei zeigten Patienten ohne Kognitionsstörungen keine subkortikalen Veränderungen, Patienten mit leichten Verhaltens- oder Kognitionsstörungen vor allem Veränderungen des Hippokampus, und Patienten mit komorbider FTD eine deutliche Beteiligung aller subkortikaler Areale. Zudem war das hippokampale Volume gemeinsam mit dem Volumen des Thalamus ein sensitiver Prädiktor der einzelnen kognitiven Phänotypen.

Als Ergänzung zu den gezeigten strukturellen Veränderungen innerhalb des Temporallappens aus den Studien 2 und 3, widmete sich die letzte Studie der vorliegenden Arbeit der Untersuchung funktioneller Veränderungen mittels funktioneller MRT in Ruhe. Die Daten wurden dabei mit Hilfe eines Graph-basierten Ansatzes analysiert, dessen Vorteil eine höhere Auflösung der Veränderungen auf Voxel-Ebene ist. Die Ergebnisse zeigen, dass es bei Patienten mit ALS neben Konnektivitätsreduktionen im Motorkortex auch zu deutlichen Veränderungen der funktionellen Konnektivität in extra-motorischen Arealen kommt. Besonders der temporo-occipitale Kortex war gekennzeichnet durch großflächige Konnektivitätsverminderungen. Es waren vor allem jene Areale betroffen, bei denen Patienten

mit frontotemporaler Demenz Konnektivitätsminderungen aufweisen. Das ist vor allem vor dem Hintergrund interessant, dass in der hier durchgeführten Untersuchung ALS Patienten ohne größere Kognitionsstörungen eingeschlossen wurden. Die hier präsentierten Ergebnisse deuten demnach daraufhin, dass das angenommene Kontinuum zwischen ALS und FTD bestehen könnte und sich auch in pathologischen Veränderungen des Temporallappens äußert. Die Kombination der hier vorgestellten Studien konnte zeigen, dass diese Veränderungen nicht nur auf der Verhaltensebene deutlich werden, sondern sich auch in strukturellen und funktionellen Veränderungen des Gehirns widerspiegeln.

Summary

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of motor neurons within the spinal cord and corticospinal tract. The heterogeneity in clinical phenotypes is large, manifesting in varying degrees of upper and lower motor neuron involvement, sporadic or familial forms, and behavioral and cognitive deficits. By now, it is well known that ALS is a multisystem disease with significant extra-motor involvement, with such involvement being reported primarily in the frontal and parietal lobes. Although there is compelling evidence for the co-occurrence of ALS and frontotemporal dementia (FTD), and temporal lobe pathology plays a crucial role in FTD, only few studies have investigated ALS-related changes in the temporal lobes. Therefore, the following work was designed to characterize temporal lobe involvement in ALS on the behavioral, structural, and functional level. By comparing the memory profile of patients with ALS with that of patients suffering from pre-Alzheimer's Disease (i.e., amnestic mild cognitive impairment, aMCI), Study 1 revealed a significant deficit in verbal memory function in ALS that was differed from that observed in the aMCI group. Whether these deficits arise from a structural deficit was addressed in Study 2, in which ALS-related volume and shape alterations in a region primarily involved in memory, namely the hippocampus, were investigated. Using high-resolution structural magnetic resonance imaging, this study showed a global hippocampal volume loss in ALS is accompanied by local shape deformations in the CA1 region in the hippocampal head. Whereas studies 1 and 2 specifically focused on memory function and structure, Study 3 investigated subcortical structures that are more generally associated with changes in behavior and cognition, such as the striatal structures, the hippocampus, the nucleus accumbens, and the amygdala, in relation to the patients' cognitive phenotype. This study demonstrated that hippocampal volume, together with the thalamic volume, differentiates best between the different cognitive phenotypes of ALS. It further revealed a gradient of subcortical grey matter pathology associated with neuropsychological changes, thus highlighting the contribution of the temporal lobe to the cognitive profile of the patients. To expand upon the results of the previous studies, study 4 investigated functional changes within the temporal lobe, using functional MRI at rest in conjunction with a graph theoretical approach. Extensive patterns of reduced functional connectivity along the temporo-occipital cortex were identified, that were similar to the pattern of degeneration usually observed in frontotemporal dementia. Studies 1-4 suggest that the temporal lobe is significantly affected in ALS, and these results therefore bolster the theory that there is a single disease continuum between ALS and FTD.

Abbreviations

AI	Asymmetry index
aMCI	Amnestic mild cognitive impairment
AD	Alzheimer's Disease
ALS	Amyotrophic Lateral Sclerosis
ALS-FTD	Amyotrophic Lateral Sclerosis – Frontotemporal dementia
ALSFRS-R	ALS Functional rating scale revised
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BDI-II	Beck Depression Inventory II
bi	Behavioral impaired
BOLD	Blood oxygen level dependency
BOSU	Bogenhausen Semantic Testbattery
bvFTD	Behavioural variant FTD
CA	Cornu ammonis
ci	Cognitive impaired
CSF	Cerebrospinal fluid
DG	Dentate gyrus
DLPFC	Dorsolateral prefrontal cortex
DNA	Deoxyribonucleic acid
DOF	Degrees of freedom
DTI	Diffusion tensor imaging
e.g.	for example
EPI	Echo planar imaging
Ex	Executive impaired
FAST	FMRIB's automated segmentation tool
FIRST	FMRIB's integrated registration & segmentation tool

FLAIR	Fluid attenuated inversion recovery
FLIRT	FMRIB's linear image registration tool
fMRI	Functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional MRI of the Brain
FNIRT	FMRIB's non-linear image registration tool
FrSBe	Frontal Systems Behavior Scale
FSL	FMRIB's Software Library
FTD	Frontotemporal dementia
FUS	Fused in sarcoma
FWHM	Full width half maximum
GM	Gray matter
GRE	Gradient recall echo
HC	Healthy controls
kDA	Kilodalton
LMN	Lower motor neuron
LONI	Laboratory of Neuro Imaging
lv-PPA	Logopenic variant PPA
MNI	Montreal Neurological Institute
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
na	not applicable
NCCI	Non-classifiable cognitive impairment
nci	non-cognitive impaired
NECI	non-executive cognitive impairment
nfv-PPA	non-fluent variant PPA
OFC	Orbitofrontal cortex
PD	Parkinson's Disease

PDC	Parkinson-dementia complex
PFC	Prefrontal cortex
PLS	Primary lateral sclerosis
PMA	Progressive muscular atrophy
PPA	Primary progressive aphasia
RCFT	Rey complex figure test
RNA	Ribonucleic acid
ROI	Region of interest
RWT	Regensburg verbal fluency test
SBM	Surface-based morphometry
SD	Standard deviation
SOD1	Superoxide dismutase 1
sv-PPA	Semantic variant PPA
TARDBP	TAR DNA-binding protein
TDP-43	Transactive response DNA binding protein 43 kDA
TE	Echo time
TFCE	Threshold free cluster enhancement
TI	Inversion time
TIV	Total intracranial volume
TMT	Trail Making Test
TR	Repetition time
UMN	Upper motor neuron
VBM	Voxel based morphometry
VLPFC	Ventrolateral prefrontal cortex
VLMT	Verbal Learning and Memory Test
WM	White matter
WMS-R	Wechsler Memory Scale revised

"Let us keep looking in spite of everything. Let us keep searching. It is indeed the best method of finding, and perhaps thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same we must give this patient today."

- Jean-Martin Charcot

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting both the upper and lower motor neurons in the cortex, brain stem, and spinal cord. The disease was first described by the French neurologist, Jean-Martin Charcot, who presented a case with gradual progressive muscle weakness and increased muscle tone (Charcot, 1865). He established the term "sclerose laterale amyotrophique" (Charcot, 1874) based on his pathological examinations in which he found sclerotic parts outside of the spinal cord (lateral sclerosis), and cell loss in the anterior horn (amyotrophy). Although Charcot was the first to define the characteristics of ALS, there were even earlier case reports from Aran, Cruveilhier and Duchenne de Boulogne who described similar symptoms of upper and lower motor neuron deficits but failed to name them as a single disease entity. The following chapter will give an overview on the epidemiological, clinical, pathogenetic, neuropsychological, and imaging evidence that has emerged since the early clinical descriptions of the disease.

1.1. Epidemiology

Population-based studies have determined the incidence rate of ALS in Europe between 2.0 to 2.3 per 100000 people per year, with men being more likely to be affected than women (3.0 vs. 2.4 per 100000 people). The incidence of ALS increases after the age of 44 years (Logroscino et al., 2008), and its incidence of onset peaks between 65-69 years for women and 70-74 years for men (Logroscino et al., 2010) and a decreasing incidence curve thereafter. Thus, age can be considered one risk factor among others for developing ALS, but unlike other neurodegenerative diseases such as Alzheimer's Disease (AD) or Parkinson's Disease (PD), it is not the primary risk factor (Al-Chalabi and Hardiman, 2013). The disease is relentlessly progressive with an average survival of about 2-3 years from symptom onset, but the variability of survival is high and depends on age of onset, site of onset, genotype, and the presence of cognitive deficits (Al-Chalabi and Hardiman, 2013). Recent population-based studies of minority populations of Europe and North America report that the frequency rate of

ALS is reduced among African, Asian, Hispanic (Cronin et al., 2007), and admixed ethnicities (Zaldivar et al., 2009), but the phenotypes seem similar across populations. Thus, these data suggest that populations that share a more common gene pool may have a higher susceptibility to the disease due to a combination of rare "at-risk" genes and local founder effects, as has been observed in genetically isolated populations of Sardinia, Ireland, or Finland (Al-Chalabi and Hardiman, 2013). Additionally, there are three foci in the Western Pacific, Japan and West New Guinea with a particular high incidence of ALS, often associated with the Parkinson-dementia complex (PDC). Although several studies have explored the influence of eating-habits and environmental factors to explain the 50 to 100 times higher frequency rates in these areas, the cause still remains unknown.

1.2. The clinical spectrum

The spectrum of clinical symptoms observed in disorders of the motor system relies on the degree of upper and lower motor neuron damage in the brain, brainstem, and spinal cord. The clinical case descriptions in the late 19th century of isolated atrophy in the anterior spinal root presented by Aran and Duchenne, in addition to Charcot´s case of pure sclerosis of the lateral spinal cord column (Charcot, 1865), and the combined pathologies in his later case series of 1874 (Charcot, 1874), laid the foundation for a detailed description of the function of the motor system and its related disorders (Goetz et al., 1995). Charcot concluded that the motor system must be organized in a two-part division: the lower motor neurons in the brainstem nuclei and anterior horn of the spinal cord being responsible for the direct innervation of the musculature; and the upper motor neurons, originating in the primary motor cortex (Betz cells) with the main outputs being the anterior corticospinal tracts, projecting to the spinal cord to innervate the lower motor neurons via interneurons (Figure 1). The recognition of a spectrum of different motor neuron symptoms was initially characterized by Gower in 1886 (Turner and Swash, 2015), and such symptoms were later classified with the term, "motor neuron disease", by Brain, in 1933 (Eisen and Shaw, 2007). Today, the main phenotypic presentations of motor neuron diseases include amyotrophic lateral sclerosis (also known as Charcot's or Lou Gehrig's Disease), progressive muscular atrophy (PMA), flail arm, flail leg, primary lateral sclerosis (PLS), and upper motor neuron dominance (Swinnen and Robberecht, 2014). It is also possible that one condition merges into the other with on-going

disease progression. The following section gives a more detailed description of the existing clinical phenotypes.

1.2.1. Amyotrophic lateral sclerosis

The clinical hallmark of pure amyotrophic lateral sclerosis is the presence of both upper and lower motor neuron signs. The majority of patients present with spinal onset ALS, where asymmetric weakness in either the arms or legs is typically the first sign of the disease. Lower motor neuron (LMN) signs include weakness, muscle atrophy, fasciculations, and hyporeflexia or areflexia, whereas upper motor neuron (UMN) damage is reflected in spasticity, a positive Babinski sign, pseudobulbar effect, exaggerated jaw or gag reflex, and hyperreflexia. In about 20% of the patients, the symptoms start in the bulbar region, resulting in slurred speech, swallowing difficulties, and fibrillations in the tongue (Swinnen and Robberecht, 2014). The variability of the presence of upper and lower motor neuron signs between patients is rather high, and causes difficulties in clinical trials. In order to address this problem, the El Escorial criteria were introduced in 1994 (Brooks, 1994), with revision in 2000 (Brooks et al., 2000). These criteria are generally used to define the involvement of upper and lower motor neuron disease in 4 regions, i.e., the bulbar region in the brainstem, and the cervical, thoracic, and lumbosacral segments of the spinal cord. A patient is classified as 'possible ALS' when there are signs of UMN and LMN damage in one of the four regions, as 'probable ALS' when there are signs of UMN and LMN damage in two of the four regions, and as 'definite ALS' when there are signs of UMN and LMN damage in at least three of the four regions. However, the clinical diagnosis of ALS does not rely on these criteria, but rather on the neurological examination, electrophysiological measures, and on the progress of the symptoms.

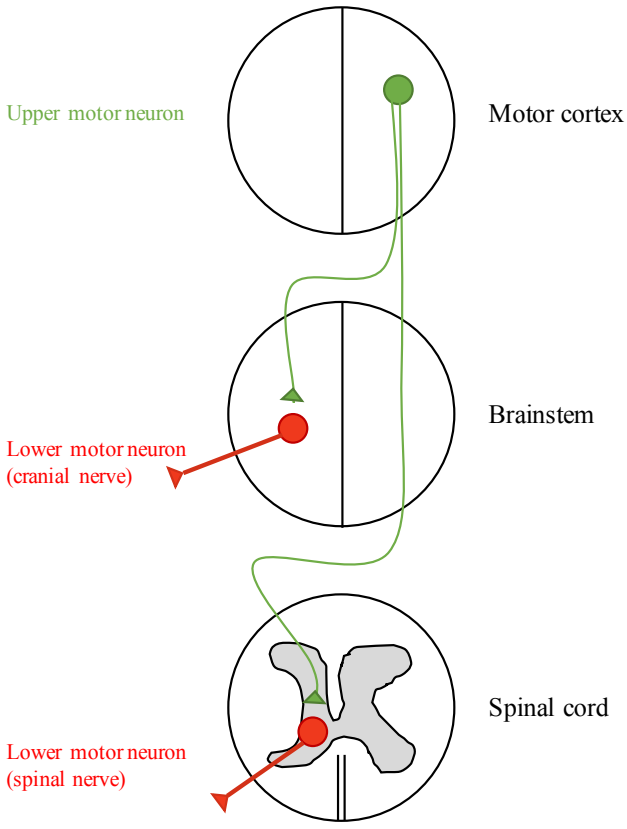

Fig. 1: Schematic representation of the motor system

1.2.2. Lower motor neuron variants

Lower motor neuron variants include progressive muscular atrophy (PMA), as well as the flail arm and flail leg phenotypes. PMA is characterized by isolated lower motor neuron involvement with no upper motor neuron damage. It differs from spinal muscular atrophy, an autosomal recessive disease, in that it has a faster disease progression and an asymmetric onset (with respect to laterality) of the disease. There is an on-going debate about whether PMA is a single disease entity or forms part of a clinical continuum (Swinnen and Robberecht, 2014), with recent clinical (Cervenakova et al., 2000; Raaphorst et al., 2011), neuroimaging (Prudlo et al., 2012; van der Graaff et al., 2011), and post-mortem (Ince et al., 2003) studies supporting the latter hypothesis. The flail limb phenotype includes both the flail arm and the less common flail leg variant, where the symptoms are limited to the respective limb for at least 12 months (Wijesekera et al., 2009). With disease progression, most of the patients develop further lower and additional upper motor neuron damage, thus fulfilling the criteria for classical ALS, but having a slightly longer survival.

1.2.3. Upper motor neuron variants

Patients presenting with isolated upper motor neuron involvement for a period of at least four years (Gordon et al., 2006), are considered to have primary lateral sclerosis (PLS). If the condition of PMA is thought to represent one end of a clinical spectrum of upper and lower motor neuron involvement, PLS is thought to represent the opposite pole. PLS is a rather rare condition of motor neuron diseases, with only 5% of patients fulfilling the Gordon criteria (Gordon et al., 2006). At the beginning, the condition is hard to differentiate from the upper motor neuron dominant phenotype, but with disease progression the latter will present with equal lower motor neuron involvement whereas the PLS patients remain stable with clinically isolated UMN signs and a far better prognosis of a mean survival of 6 years (Gordon et al., 2006).

1.3. Genetics

Since the first description of the disease, the identification of pathogenic mechanisms has been challenging, due to the large heterogeneity in clinical phenotypes. Several environmental factors contributing to ALS have been discussed in the literature, such as physical activity,

smoking, heavy metals, chemicals, or geographical clustering, but the lack of clear models and hypotheses makes it difficult to establish a link between external causative factors and ALS pathology (for current review see Al-Chalabi and Hardiman, 2013 and Paez-Colasante et al., 2015). In contrast to environmental factors, the identification of several genetic mutations in ALS pedigrees, as well as sporadic ALS cases, has provided more insight into the possible mechanisms of disease (Paez-Colasante et al., 2015). The following section will review the most common mutations and their suggested role in ALS pathogenesis. A detailed review of current genetic mutations can be found in Paez-Colasante et al. (2015), Renton et al. (2014), and Marangi and Traynor (2015).

1.3.1. Superoxide dismutase 1 (SOD1)

Amyotrophic lateral sclerosis is generally known to be a non-hereditary disease although there are several reports scattered throughout the literature on familial aggregation of ALS (Kurland and Mulder, 1955a). In 1955, Kurland and Mulder reviewed cases of familial ALS over the previous 100 years. Combining these findings with their own data from families living in Guam, they came to the conclusion that, "typical amyotrophic lateral sclerosis in familial aggregation is not as rare as was formerly believed and that such cases when they do occur cannot be distinguished clinically or pathologically from the sporadic cases" (Kurland and Mulder, 1955b). It took another four decades until mutations in the superoxide dismutase gene (SOD1) on chromosome 21 were linked to familial ALS (Rosen, 1993). Superoxide is rather toxic and causes oxidation of cell constituents, whereas the superoxide dismutase (SOD) enzyme catalyzes the partitioning of the superoxide radical into either molecular oxygen or hydrogen peroxide. SOD can be differentiated into three subtypes; SOD1 is located in the cytoplasm, SOD2 is located in the mitochondria, and SOD3 is located in the extracellular space. The SOD1 protein uses copper (Cu) and zinc (Zn) as a binding cofactor and is therefore called Cu/Zn superoxide dismutase. The majority of SOD1 mutations are missense mutations leading to the degeneration of motor neurons. This was believed to be a consequence of a toxic gain of function, however, recent studies suggest that motor neuron death is caused by noxious SOD1 protein aggregations and mitochondria dysfunction (Paez-Colasante et al., 2015). To date, there are about 170 identified SOD1 mutations that account for 12-20% of familial ALS cases and 1-2% of sporadic ALS cases (Marangi and Traynor, 2015).

1.3.2. TAR DNA-binding protein (TARDBP) and Fused in Sarcoma (FUS)

The TARDBP gene on chromosome 1 encodes the TAR DNA-binding protein 43 kDA (TDP-43) that is a protein containing 414 amino acids, and it is linked to many aspects of RNA-related metabolism (Ling et al., 2013). TDP-43 is predominantly located in the nucleus (Buratti et al., 2001), shuttles between the nucleus and cytoplasm (Ayala et al., 2008), and is primarily expressed in tissues such as the brain, muscles, and inner organs (Buratti et al., 2001). In ALS, as well as in ubiquitin-positive, tau-negative frontotemporal dementia (FTD), pathological protein aggregations of TDP-43 were found to be abnormally ubiquitinated (Neumann et al., 2006), hyper phosphorylated (Hasegawa et al., 2008), and mainly located near the carboxyl-terminal region (Neumann et al., 2009). The discovery of ubiquitin-positive TDP-43 inclusions in both ALS and FTD patients in 2006 (Neumann et al., 2006) was followed by the identification of ALS-related mutations in the TARDBP gene, accounting for 4% of familial and 1.5% of sporadic ALS cases (Mackenzie et al., 2010).

Shortly after recognizing TARDBP mutations as one cause of abnormal TDP-43, mutations on another gene involved in RNA metabolism, namely the FUS gene, were identified in a small proportion of familial (5%) and sporadic (1%) ALS patients. FUS is located on chromosome 16 and encodes the FUS/TLS protein (Fused in Sarcoma/ Translocated in Sarcoma), which shares functional homology with TDP-43 (Marangi and Traynor, 2015). FUS missense mutations have been found in both familial and sporadic ALS, as well as in FTD and affect mainly the C-terminus of the protein (Kwiatkowski et al., 2009; Vance et al., 2009). There are no phosphorylated TDP-43 (pTDP-43) inclusions in ALS patients with FUS mutations. This is particularly of interest since TDP-43 inclusions have been found in over 90% in the motor neurons of both sporadic and familiar ALS (Ling et al., 2013). The fact that ALS patients with FUS mutations present with FUS-immunoreactive cytoplasmic inclusions instead, suggests that FUS pathology is independent of pTDP-43 accumulation (Vance et al., 2009).

1.3.3. C9orf72

Recent advances in genome sequencing techniques have led to further identification of disease modifying genes. In 2011, two independent research teams identified a mutation in the C9orf72 gene on chromosome 9 in families with ALS, FTD, and ALS-FTD in Finland

(Renton et al., 2011) and the USA (DeJesus-Hernandez et al., 2011). Because these groups used a genome-wide association approach they were able to establish a genetic link between these conditions (see also 1.4.7). The C9orf72 gene is located on the short arm of chromosome 9 open reading frame 72 and encodes the C9orf72 protein. The pathogenic variant consists of GGGGCC hexanucleotide repeat expansions in the promoter or in intron 1, depending on the three different gene transcript variants (Rohrer et al., 2015). Repeat expansions between 2 and 20 are found within the normal population, but expansions ranging from 30 to more than a few hundred are considered pathogenic. Patients carrying an intermediate state of repeat expansions (between 20 and 30 repeats) are thought to have similar features to those with repeat expansions greater than 30 (Byrne et al., 2014). Similar to carriers of mutations in TARDBP, C9orf72 mutation carriers show also phosphorylated TDP-43 aggregations, albeit inclusions differ in that they contain aggregated dipeptide repeat proteins (Ash et al., 2013; Mori et al., 2013). To date, the pathogenic repeat expansions are the major genetic cause of ALS and FTD, accounting for a large proportion of familial cases and a considerable amount of sporadic cases in populations of European descent (Rohrer et al., 2015).

1.4. Neuropsychology

Early descriptions of patients with motor neuron disease in the late 19th century focused solely on the characteristics of motor dysfunction and until recently it was the common assumption that ALS only affects the motor system. Studies from the last two decades, however, have demonstrated that about 50% of patients show a range of cognitive and behavioral deficits, such as executive dysfunction, memory deficits, language impairment, apathy, disinhibition, or impaired social cognition. The following section reviews the most commonly observed neuropsychological deficits in each cognitive domain, and gives an overview of suggested cognitive phenotypes in ALS.

1.4.1. Executive function

The term "executive function" encompasses a broad range of cognitive processes, including fluency and flexibility of verbal and figural contents, the generation of strategies for the solution of novel problems, as well as planning and the regulation of behavior (Lezak, 2004).

Executive deficits have been associated with frontal lobe damage, as case reports observed that lesions within this part of the brain led to changes in behavior and cognition. Anatomically, the human frontal lobe comprises about 40% of the total cortex, and it is highly interconnected with other cortical and subcortical regions. The main functional subdivisions within the frontal lobe are the motor (and premotor) cortex, and prefrontal cortex (PFC), both of which can be subdivided into functionally distinct regions. The PFC is divided into the dorsolateral (DLPFC) and ventrolateral (VLPFC) cortex, the orbitofrontal cortex (OFC), and the medial frontal cortex (Wood and Grafman, 2003). Although reports from patients with PFC lesions suggest a distinctive functional role for each of these three subregions (Badre and D'Esposito, 2009), all of them are involved when top-down control is needed in non-automated processes (Miller & Cohen, 2001). Several theories on the nature of executive function and the involved prefrontal regions have been proposed in the literature since the 1950, including the "cognitive control" model by Posner and Snyder (Posner and Snyder, 1975), the "central executive" hypothesis by Baddeley and Della Sala (Baddeley and Della Sala, 1996), the "integrative model" by Miller and Cohen (Miller and Cohen, 2001), and the "cascade of control" model by Banich (Banich, 2009). Overall, there is no universal definition for executive function (for overview see Goldstein and Naglieri, 2013) and several neuropsychological tests have been proposed to assess different aspects of the variety of high-level cognitive abilities. The most frequently used tests include measures of planning (Tower of London), verbal fluency (Regensburger verbal fluency test), working memory, response inhibition (Stroop test), and set-shifting (Trail Making Test, Wisconsin Card Sorting Test).

Large population-based studies report executive dysfunction as the most frequently observed cognitive deficit in ALS (Massman et al., 1996; Montuschi et al., 2015; Phukan et al., 2012; Ringholz et al., 2005). In particular, letter and category fluency are among the most sensitive markers to detect cognitive dysfunction (Goldstein and Abrahams, 2013; Phukan et al., 2007), even when accounting for speech impairment (Abrahams et al., 2000). These measures are thought to be associated with DLPFC dysfunction (Abrahams et al., 1996; Abrahams et al., 2004). Throughout the literature there are also reports on impaired set-shifting (Abrahams et al., 1997; Kasper et al., 2015; Moretti et al., 2002), as well as reduced working memory capacity, affecting specifically the "central executive" component (Abrahams et al., 2000; Volpato et al., 2010). Executive dysfunction in non-demented ALS patients has been associated with the bulbar phenotype, a faster disease progression, and shorter survival

(Elamin et al., 2011), underscoring the relevance of the necessary assessment of cognitive function in the disease.

1.4.2. Memory

Memory refers to the capacity to encode, retain, and retrieve essential information for everyday living. Although intact functioning of multiple brain regions is needed for proper memory processing, it is mainly associated with the temporal and frontal lobes. There are four major categories in which memory can be subdivided: working memory, the perceptual representation system, semantic memory, and episodic memory (Tulving, 2000). The neuropsychological assessment of memory is determined through tests that typically include the learning of new material (encoding) usually presented in the form of words, objects, pictures or sentences, a retention interval (storage) eventually filled with other cognitive tasks, and reporting that which was previously learned (retrieval) after a given time period. In contrast to the well-documented executive deficits in ALS, studies investigating memory impairment show more inconsistent results. A recent meta-analysis revealed consistent results among studies in immediate verbal memory recall (retrieval after a short interference task) with a heterogeneity of 20%, while impairment in delayed verbal recall performance was reported with a much higher variability (heterogeneity of 56%) (Beeldman et al., 2015). Although retrieval performance is associated with both the prefrontal and medial temporal lobes (MTL), immediate recall relies primarily on mid-dorsolateral frontal cortical function (Petrides et al., 1993), and delayed recall performance depends mainly on intact functioning of structures of the MTL, such as the hippocampus.

1.4.3. Language

Language skills encompass a wide variety of different functions. They are highly intertwined with motor function, attention, executive function, and perception, thus making the assessment rather complex and extensive. Language is primarily associated with intact left hemispheric function, although bilateral or right hemispheric representations are also reported. On a functional level, language can be separated into expressive language, which is strongly associated with the left posterior frontal cortex (Broca's area), and perceptive language, typically related to the left posterior temporo-parietal cortex (Wernicke's area). Assessment of language function in ALS has not received much consideration during the last

decades as it is highly influenced by the patients' bulbar symptoms, respiratory problems, and executive dysfunction, and it can therefore be a secondary symptom of a primary problem. Only recently the focus has shifted towards a more thorough testing of language in ALS, as a recent study suggests that it is as common as executive dysfunction and in part dissociable from it (Taylor et al., 2013). Observed deficits include a variety of language functions including confrontation naming, syntactic processing, and comprehension, as well as verb processing (Ash et al., 2015; Leslie et al., 2015; Phukan et al., 2012; Taylor et al., 2013; Tsermentseli et al., 2015).

1.4.4. Visuo-spatial skills

Visuo-spatial skills are a relevant feature of constructional function, a concept that incorporates two main factors: drawing and building. Impairment of these functions is thought to affect a number of everyday activities such as driving, placing dishes in a dishwasher, and meal planning. Assessment of visuo-spatial skills is rather complex as it needs to be differentiated from pure perceptional deficits, spatial confusion, reduced attention, and motor planning difficulties (Fischer and Loring, 2004). Typical construction tasks include copying a complex figure (e.g., Rey Complex Figure Test, Osterrieth, 1944), or the reproduction of block constructions in the Block Design task of the Wechsler Adult Intelligent Scale (e.g., WAIS-IV, Petermann, 2012). Studies on patients with unilateral brain lesions and impaired visuo-construction suggest that right hemispheric lesions are often associated with a fragmented approach during copying the complex figure while not capturing the overall "gestalt". On the contrary, patients with left hemispheric lesions are more often reported to omit details of the "gestalt" and sloppy copying (Fischer and Loring, 2004) while capturing the right proportions of the figure. Visuo-spatial function is seldom reported to be deficient in ALS (Raaphorst et al., 2011; Ringholz et al., 2005), although it seems to be an especially sensitive measure for cognitive change during disease progression (Elamin et al., 2013).

1.4.5. Behavior

Similar to changes in cognition, changes in behavior have been somewhat neglected in patients with ALS as they are often thought to be hidden by the patients' motor disability. With the recognition of a clinical spectrum between the conditions of ALS and FTD (see 1.3.

and 1.4.7), research in both fields has expanded towards the investigation of features that are commonly observed in the other disease. In FTD, a common feature of the disease are changes in behavior and personality that play a critical role in patients' outcome and management. Behavioral changes include the loss of empathy, disinhibition, perseverative and stereotyped behavior, aggressive actions, apathy, and hyperorality, although they do not necessarily occur together. Anatomically, these changes are primarily associated with dorsolateral prefrontal, orbitofrontal, and medial prefrontal dysfunction and are often related to executive functions.

ALS-related deficits in behavior are mainly assessed by standardized behavioral questionnaires or interviews with the caregiver (e.g., Frontal Systems Behaviour Scale, Grace and Malloy, 2001), the Neuropsychiatric Inventory, Cummings et al., 1994), or ALS specific questionnaires that have been developed to minimize a confounding effect of motor disabilities (ALSFTD-Q, Raaphorst et al., 2012), Mind-B (Mioshi et al., 2014b), Apathy Scale (Radakovic et al., 2016). Apathy is among the most common behavioral symptoms (Lillo et al., 2011; Mioshi et al., 2014b), but disinhibition, aberrant eating behavior, stereotypies, and compulsions have been also described (Gibbons et al., 2008; Grossman et al., 2007), although such behavioral disturbances appear to be less frequent. It has been shown that several of these behavioral symptoms can appear early during the disease course and even precede motor symptoms (Mioshi et al., 2014a). The presence of moderate to severe apathy is associated with shorter survival (Caga et al., 2016) and does impact the caregivers' quality of life (Chio et al., 2010).

1.4.6. Cognitive phenotypes

The previous sections demonstrated that there is a broad range of possible cognitive and behavioral deficits in ALS, adding to the heterogeneous clinical (see section 1.2) and genetic phenotypes (see section 1.3) of the disease. Several approaches have been suggested for the classification of cognitive and behavioral deficits, beginning with the consensus criteria in 2009 (Strong et al., 2009). Within this framework, four axes have been proposed to define the motor neuron disease variant (axis I), the cognitive and behavioral dysfunction (axis II), additional non-motor manifestations (axis III), and disease modifiers (axis IV). Axis II includes pure ALS without cognitive or behavioral deficits, ALS with cognitive impairment (ALSci), ALS with behavioral impairment (ALSbi), and ALS with comorbid frontotemporal

dementia (ALS-FTD). To be classified as ALSbi, patients have to meet at least two non-overlapping criteria regarding behavioral changes, such as perseverative and stereotyped behavior, hyperorality, loss of insight, disinhibition, apathy, or impulsiveness (Strong et al., 2009). To meet criteria for ALSci, patients have to score at or below the 5th percentile on at least two independent tests measuring executive function. The ALS-FTD phenotype comprises all clinical subtypes of FTD (see 1.4.7).

As outlined in the previous sections, cognitive impairment in ALS includes more than just executive dysfunction. Several population-based studies investigated the frequency and pattern of cognitive impairment and extended the concept of the consensus criteria (Strong et al., 2009). Based on their findings in a cohort of 160 ALS patients, Phukan and colleagues extended the Strong criteria by suggesting a domain-based classification into four groups according to their cognitive performance: ALS without cognitive impairment; ALS with impairment in executive function (ALS-Ex); ALS with non-executive cognitive impairment (ALS-NECI); ALS with comorbid frontotemporal dementia (ALS-FTD) (Phukan et al., 2012). This was complemented by the idea of whether the impairment was limited to one domain, such as language, or to multiple domains (e.g., executive function and memory). Based on this classification approach, 47% of the patients showed no cognitive impairment, 21% showed executive dysfunction, 14% were impaired in a non-executive domain, and 14% fulfilled criteria for ALS-FTD. Montuschi and colleagues extended this model by adding the categories “ALS with behavioral impairment” (ALS-bi) and “ALS with non-classifiable cognitive impairment” (ALS-NCCI, Montuschi et al., 2015). In their cohort of 183 patients, 20% fulfilled criteria for ALS with executive impairment (ALS-ECI), 6% for ALS-NECI, 13% for ALS-FTD, 6% for ALS-bi, while 50% were cognitively and behaviorally normal and 6% showed non-classifiable cognitive impairment.

1.4.7. ALS and FTD as a spectrum disorder

ALS and FTD were traditionally considered as two different neurological disorders with diverging clinical symptoms when they were first described in the late 19th century. Since then, a considerable amount of case reports on patients with ALS who also show signs of FTD and vice versa has appeared in the literature. As described previously in section 1.4.6, current population-based investigations have found that about 13-15% of patients with ALS fulfill the criteria for FTD (Montuschi et al., 2015; Phukan et al., 2012), and about the same

proportion of patients with FTD shows motor dysfunctions (Burrell et al., 2011; Lomen-Hoerth et al., 2002). The recognition that there are ubiquitin-positive TDP-43 inclusions in both ALS and FTD (see 1.3.2) and a shared pathogenic variant of the hexanucleotide repeat expansion on chromosome 9 in families with ALS, FTD, and ALS-FTD has further bolstered the hypothesis of a clinicopathological spectrum between the diseases. FTD is the second most common type of dementia with an incidence of 3.5-4.1 per 100000 people between the age of 45-65 years (Knopman et al., 2004; Mercy et al., 2008). It is characterized by the selective involvement of the frontal and temporal lobes, and comprises a variety of different phenotypes, such as bvFTD (Rascovsky et al., 2011), semantic variant PPA (sv-PPA), non-fluent/agrammatic variant PPA (nfv-PPA), and logopenic variant PPA (lv-PPA, Gorno-Tempini et al., 2011). Although all variants can occur with ALS, it is the bvFTD phenotype that is most frequently observed in ALS-FTD.

In contrast to the clinical phenotypes, where a transition from one condition into the other is frequently observed with disease progression, it is yet unknown if the cognitive phenotypes lie on one clinical continuum where ALS and FTD lie on opposite poles, or if they represent separate disease entities. A recent longitudinal studies that has been conducted to evaluate the temporal evolution of the patients' cognitive status demonstrated that patients who were cognitively intact in the beginning remained without deficits over a period of at least six months. In contrast, for patients who initially presented with cognitive deficits, it was more likely the case that previously unaffected functions became deficient during follow-up, namely delayed recall of visual memory and naming performance (Elamin et al., 2013). Notably, only one patient out of 186, who had overt behavioral deficits in the beginning, later fulfilled criteria for behavioral variant FTD. Results from another cohort replicated these findings, suggesting that executive dysfunction remains stable during disease progression (Kasper et al., 2016). Further long-term evaluations need to be conducted to investigate whether all patients with ALS will develop features of FTD and vice versa or if the variability in cognitive and behavioral deficits represents different disease phenotypes with no progression from one into another.

1.5. Neuroimaging

In addition to post mortem, genetic, and molecular investigations, the application of advanced neuroimaging techniques has provided detailed insight into ALS pathology *in vivo*. During the last two decades a variety of studies using magnetic resonance imaging (MRI) helped to better understand the impact of the disease on different brain regions. MRI is mainly divided into structural and functional MRI, although this separation might be problematic as structure and function are widely intertwined in the brain (Symms et al., 2004). Structural magnetic resonance imaging is a non-invasive technique for examining the anatomy and pathology of the brain. In ALS, structural MRI is mostly used to exclude any pathological processes that could mimic UMN and LMN signs, such as tumors or strokes (Turner and Verstraete, 2015), but it is not part of current diagnostic criteria (Brooks et al., 2000). Nevertheless, the application of advanced structural MRI has highlighted how the disease affects the brain, and might be of interest in the future as a possible outcome measure in clinical trials. Functional magnetic resonance imaging (fMRI) is used to study human brain function and is primarily assessed by blood oxygenation level dependent (BOLD) contrast imaging. A distinction is made between task-based and resting-state fMRI (rsfMRI) experiments, both of which have been applied in ALS research in order to investigate functional changes associated with the disease.

The following section reviews findings of both structural and functional imaging studies, particularly in the light of different clinical, genetic, and cognitive phenotypes in ALS.

1.5.1. Structural imaging

Upper and lower motor neuron signs are the hallmark of classical ALS; thus numerous studies investigated motor-related structural changes using voxel- and surface-based approaches or diffusion tensor imaging (DTI). The majority of studies using voxel-based morphometry (VBM) reported gray matter volume loss in the motor cortex (Bede et al., 2013a; Chang et al., 2005; Chen and Ma, 2010; Grosskreutz et al., 2006) although results vary particularly with regard to lateralization and extra-motor involvement, probably as a result of heterogeneous group samples and different usages of the methodology (Verstraete and Foerster, 2015). Findings from surface-based approaches, however, show far more consistent results of cortical thinning in primary motor regions (Agosta et al., 2012; Schuster et al., 2013; Verstraete et al., 2012). Studies taking advantage of diffusion tensor imaging demonstrated

changes in water diffusion parameters in the corticospinal tract and corpus callosum that are thought to arise from axonal degeneration and white matter disorganization (Verstraete and Foerster, 2015). Common metrics derived from the tensor model include the radial diffusivity, mean diffusivity, and fractional anisotropy (FA). FA is an indicator of the overall directionality of white matter bundles and it appears to be among the most sensitive parameters that can be used to map ALS-related diffusivity changes in the corpus callosum and corticospinal tract (Agosta et al., 2010; Cardenas-Blanco et al., 2014; Thivard et al., 2007). The usage of FA values within graph-based approaches revealed decreased structural connectivity not only within primary motor regions but also between the motor cortices and supplemental motor areas (Verstraete et al., 2011). Investigating the structural network in the course of the disease revealed an expanding loss of network structure spreading from primary motor areas to frontal and parietal regions (Verstraete et al., 2013). Recently, FA values have also been used to replicate a neuropathological staging system for pTDP-43 spread (stages I-IV) (Brettschneider et al., 2013), showing that individual staging based on *in* vivo imaging data is possible, although not in every patient (Kassubek et al., 2014). These findings have been supported by computational simulation of disease spread along anatomical pathways (Schmidt et al., 2016) and a large-scale multicenter DTI study on 253 ALS patients (Muller et al., 2016), indicating that DTI is a promising imaging marker for mapping ALS-related structural changes.

In addition to structural changes in primary motor areas, recent studies demonstrated that extra-motor changes are also a common feature of ALS, although they are highly variable and can be related to different clinical, cognitive, and genetic phenotypes. ALS patients carrying the C9orf72 hexanucleotide mutation show a distinctive pattern of cortical, subcortical, and cerebellar involvement in comparison to non-carriers (Bede et al., 2013b; Bede et al., 2015; Bede et al., 2013c). These changes are already detectable in asymptomatic carriers (Walhout et al., 2015), suggesting that pathological processes at the cellular level happen years before the appearance of clinical signs. Apart from differences in genetic phenotypes, recent studies show that the degree of upper and lower motor neuron involvement, resulting in different clinical phenotypes (see section 1.2), is also related to structural brain changes. Walhout and colleagues demonstrated that cortical thinning within the primary motor cortex is mainly dependent on the degree of upper motor neuron involvement since patients with PMA or ALS mimics did not show atrophy within this region (Walhout et al., 2014). A similar picture emerged when patients were segregated according to their site of onset, showing that the

bulbar phenotype is associated with greater central white matter degeneration than the limb phenotype (Cardenas-Blanco et al., 2014). With increasing awareness for cognitive and behavioral deficits in ALS, only a few studies have investigated structural changes with regard to cognition (Agosta et al., 2016; Kasper et al., 2014; Mioshi et al., 2013; Schuster et al., 2014; Tan et al., 2014), and these suggest a gradient of cortical grey and white matter pathology along the phenotypes of ALS, ALS-Plus, and ALS-FTD, although the involvement of cortico-subcortical networks in cognitive changes remains unknown.

1.5.2. Functional imaging

Studies taking advantage of task-based fMRI reported an increase of activation in bilateral motor areas (Schoenfeld et al., 2005; Stanton et al., 2007), and the functional recruitment of adjacent regions (Konrad et al., 2002) during motor tasks, indicative of ongoing cortical reorganization processes in patients with ALS. Resting-state fMRI investigations, however, revealed both increases (Agosta et al., 2011) and decreases (Jelsone-Swain et al., 2010; Mohammadi et al., 2009) in motor functional connectivity. Increases in motor connectivity have been observed in connections that were characterized by white matter damage (Douaud et al., 2011), leading to the assumption that connectivity increases serve as a compensatory mechanism. The majority of these analyses have primarily focused on connectivity alterations within the sensorimotor network and its relation to extra-motor regions using independent component analysis, seed based approaches, or graph theoretical approaches (Agosta et al., 2011; Fang et al., 2016; Fekete et al., 2013).

The relationship between functional connectivity alterations and different ALS phenotypes such as genetic variants or different clinical phenotypes is yet relatively unknown. A recent study reported increases in cerebro-cerebellar connectivity in patients with primary lateral sclerosis compared to controls (Meoded et al., 2015), while another found connectivity increases in asymptomatic SOD1 and C9orf72 mutation carriers (Menke et al., 2016). With respect to extra-motor involvement, recent studies have revealed rather heterogeneous results, reporting either increased (Agosta et al., 2013a; Agosta et al., 2011; Douaud et al., 2011) or decreased (Agosta et al., 2013a; Luo et al., 2012; Mohammadi et al., 2009) extra-motor functional connectivity, although the different cognitive phenotypes were not considered. The affected regions comprised mainly frontal and parietal areas with relative sparing of temporal

regions, possibly reflecting the frequently observed fronto-executive deficits in ALS (see section 1.4).

1.6. Aims of the thesis

Amyotrophic Lateral Sclerosis is a progressive disease of the motor system. Although initially thought to only affect motor function, it is now recognized as a multi-system disease. The heterogeneity in clinical phenotypes is large, manifesting in varying degrees of upper and lower motor neuron involvement; different genetic mutations; and varying behavioral or cognitive deficits (Swinnen and Robberecht, 2014). Recent findings from histopathological (Neumann et al., 2006), genetic (DeJesus-Hernandez et al., 2011; Renton et al., 2011), and neuropsychological (Phukan et al., 2012) investigations suggest a common pathology between the conditions of ALS and frontotemporal dementia (FTD), both of which share clinicopathological features. Although there is emerging evidence from behavioral and anatomical studies for extra-motor pathology in the frontal and parietal lobes in ALS, little is known about the involvement of the temporal lobes in the disease, in spite of the fact that it represents a core component of FTD pathology. For example, the occurrence of memory deficits, as a core component of temporal lobe function, is highly heterogeneous among ALS patients (Beeldman et al., 2015), and the question arises as to whether such memory deficits arise from a temporal lobe deficit or are modulated by executive dysfunction. Similarly, ALS-related structural and functional alterations in the temporal lobe are yet understudied, given the proposed overlap with FTD. Therefore, the current work aimed to characterize the involvement of the temporal lobe in amyotrophic lateral sclerosis on the behavioral, structural, and functional level.

To this end, Study 1 investigated temporal lobe function in ALS by comparing the patients' neuropsychological profile with a group of amnestic MCI patients that suffer from a core temporal lobe deficit (Machts et al., 2014). In continuation of the first study, the underlying anatomical correlates of memory as a core temporal lobe function were investigated in Study 2 by means of hippocampal volume and shape using structural magnetic resonance imaging (MRI). Besides its integrative role in memory, the hippocampus as a subcortical structure is highly interconnected with other subcortical structures, such as the amygdala and the striatum. Although the striatum is commonly associated with motor function, it is now increasingly

recognized to play a critical role in the modulation of behavior and cognition. Therefore, Study 3 was designed to explore subcortical structural changes in different, interconnected subcortical structures, and how these changes relate to the different cognitive phenotypes of ALS by using both complementary structural MRI measures and neuropsychological measures (Machts et al., 2015). Finally, in order to assess alterations at the functional level, Study 4 used fMRI measured at rest to identify ALS-related connectivity reductions in the absence of major cognitive deficits.

2. Study 1: Memory impairment in ALS is different from that observed in patients suffering from Alzheimer pathology[1]

2.1. Introduction

Study 1 was designed to characterize memory deficits in ALS and compare them to a group of patients suffering from amnestic mild cognitive impairment (aMCI). Although executive dysfunction is the most frequent reported cognitive deficit in ALS (Abrahams et al., 2000; Elamin et al., 2013; Goldstein and Abrahams, 2013; Phukan et al., 2012; Phukan et al., 2007; Raaphorst et al., 2010), there is increasing evidence that other cognitive domains such as language and memory are also affected (Abrahams, 2013; Phukan et al., 2012). Most of the studies focusing on memory dysfunction in ALS supports an encoding or short recall deficit, with relative sparing of consolidation performance (Christidi et al., 2012; Massman et al., 1996), but the findings are inconsistent and have not been related to temporal lobe dysfunction. Recently, there has been growing postmortem and *in vivo* evidence of temporal lobe involvement in ALS based on hippocampal TDP-43 pathology (Braak et al., 2013; Brettschneider et al., 2013) and volume loss (Bede et al., 2013c; Takeda et al., 2009; Takeda et al., 2007). Temporal lobe pathology is also a key feature of Alzheimer´s Disease (AD), with hippocampal atrophy even in early stages (Mueller et al., 2010). Based on the shared hippocampal involvement in both ALS and AD, we sought to investigate whether there are dissociable hallmarks for memory deficits in ALS and AD. The extent of hippocampal pathology in AD is, to date, far more extensive than in ALS making a direct comparison between ALS and AD patients difficult. Therefore, we decided to compare ALS patients to a group of patients that suffer from an AD prodromal stage, i.e., amnestic mild cognitive impairment (Winblad et al., 2004). Patients with aMCI have overt deficits in cognition, especially in the memory domain, but are still largely independent in daily activities. They have a greater risk of developing dementia than cognitively normal persons, but at the level of the individual patient, the prognosis might be variable (Knopman, 2013). Hippocampal atrophy is also a hallmark of aMCI, but it occurs at a much smaller extent than in patients with AD (Franko et al., 2013), making aMCI patients comparable to patients with ALS in

[1] The chapter is partially based on an article by Machts, J., Bittner, V., Kasper, E., Schuster, C., Prudlo, J., Abdulla, S., Kollewe, K., Petri, S., Dengler, R., Heinze, H.J., Vielhaber, S., Schoenfeld, M.A., Bittner, D.M. 2014. Memory deficits in amyotrophic lateral sclerosis are not exclusively caused by executive dysfunction: a comparative neuropsychological study of amnestic mild cognitive impairment. BMC neuroscience 15, 83. doi:10.1186/1471-2202-15-83.

terms of hippocampal degeneration. In this first study, we focused on verbal memory performance and hypothesized a qualitative difference between patients with ALS and aMCI. In addition, we investigated the extent to which memory deficits are modulated by executive dysfunction.

2.2. Methods

2.2.1. Participants

A total of 40 patients with ALS, 39 patients with amnestic mild cognitive impairment, and 40 healthy controls participated in this cross-sectional study. ALS patients were recruited consecutively from both the outpatient clinics of the departments of neurology at Otto-von-Guericke University and Hannover Medical School. Diagnosis was made in accordance with the revised El Escorial Criteria (Brooks et al., 2000), and physical disability was rated using the revised ALS rating scale (ALSFRS-R, Cedarbaum et al., 1999). Disease duration was calculated as the time between patients' subjective disease onset and the present clinical examination. The rate of disease progression was estimated based on the decline in ALSFRS-R since symptom onset in relation to patients' disease duration (48-ALSFRS-R/disease duration, Kimura et al., 2006). The following phenotypes were considered: classical ALS (n = 27), upper motor neuron dominant ALS (n = 2), progressive muscular atrophy (n = 7), flail limb (n = 3), and primary lateral sclerosis (n = 1). A concomitant diagnosis of frontotemporal dementia (FTD) was defined on current diagnostic criteria based on information given by the caregiver during clinical examination. Three patients fulfilled the criteria for comorbid behavioral variant FTD (Rascovsky et al., 2011). Amnestic MCI patients were recruited from the memory outpatient clinic at Otto-von-Guericke University Magdeburg and diagnosis was made in accordance with the revised Petersen criteria for mild cognitive impairment (Winblad et al., 2004). Healthy controls were recruited through public advertisement, screened for cognitive impairment prior to inclusion and had to score within the normal range ($\geq$ 26) of the Montreal Cognitive Assessment (MoCA, Nasreddine et al., 2005). The MoCA is a short screening instrument encompassing several cognitive domains such as executive function, memory, verbal fluency, visuo-spatial skills, and orientation. All participants filled out a self-rating depression questionnaire on the day of their neuropsychological evaluation (Beck Depression Inventory-II, Hautzinger et al., 2006). Table 1 summarizes the demographic characteristics for the ALS and aMCI patients and healthy controls. Exclusion criteria for all

participants included other neurological or psychiatric conditions, traumatic brain injury, or cerebrovascular disease.

2.2.2. Neuropsychological assessment

All participants underwent a detailed neuropsychological assessment lasting approximately 2 hours. The test battery included a range of commonly used standardized neuropsychological instruments to assess the domains of executive function, memory, and visuo-spatial skills. Executive functions were assessed by letter fluency ("K") and flexibility (alteration between "G" and "R"), as well as semantic fluency ("animals") and flexibility (alteration between "sport" and "fruit") from the Regensburger verbal fluency test (RWT, Aschenbrenner et al., 2000). The RWT assesses the spontaneous production of words under restricted search conditions during a fixed period of time (Strauss et al., 2006). In the fluency condition, subjects are asked to name as many nouns, verbs, or adjectives as possible, starting with a given letter (letter fluency) or category (semantic fluency). In the flexibility conditions, subjects have to alternate between two letters or two categories in order to assess the cognitive flexibility. The time frame for each task used in this study was 1 minute. Cognitive flexibility was also assessed using the Trail Making Test (TMT, Reitan, 1992), which additionally gives information on speed processing and attention (Strauss et al., 2006). It requires the subject to connect a sequence of randomly arranged, consecutive numbers (1-25) as quickly as possible in the first part A, whereas the second part B is slightly more difficult in terms of connecting a sequence of alternating numbers and letters in the correct order (1-A-2-B- ... -13). While the first part provides information on visual exploration and screening, the second part additionally measures cognitive flexibility. In order to rule out possible motor confounds in the ALS cohort, the ratio between part B and A was calculated for each subject. Verbal working memory was assessed by the backward digit span from the Wechsler Memory scale-revised (WMS-R, Härting et al., 2000), where subjects have to repeat a sequence of numbers in reverse order.

The battery also tested for impairment in non-executive cognitive domains. Memory functions were assessed using the Verbal Learning and Memory Test (VLMT, Helmstaedter et al., 2001), a German version of the Rey Auditory Verbal Learning Test. The VLMT tests for impairment in verbal learning, immediate and delayed recall, and recognition memory. It consists of 15 nouns that are read aloud to the subject and repeated for five consecutive trials

while obtaining the word order. After each trial, the subject is asked to repeat as many words as possible from the list. Subsequent to the fifth trial, a new list of the same length is presented and recalled to assess the proactive and retroactive interference performance. Following this, the subject is asked to recall the words from the first list without further presentation of the list (trial 6). The recall of the first list is repeated after a delay period of 20-30 minutes (trial 7) and followed by a recognition task, where words from the first list have to be discriminated from the second list and new words (Helmstaedter et al., 2001; Strauss et al., 2006). The following subtests were used for group comparisons: verbal learning (sum of trials 1-5), immediate recall (difference between trial 5 and 6), delayed recall (difference between trial 5 and 7), and recognition (corrected for false positive and interference items). Additionally, verbal short term memory was assessed using the forward digit span task from the revised Wechsler Memory Scale (Härting et al., 2000). Here, the subject is asked to repeat strings of digits of increasing length in the same order as presented by the examiner.

Visuo-spatial abilities were evaluated using the copy trial of the Rey Complex Figure Test (RCFT, Osterrieth, 1944). The subject reproduces the figure with no pre-defined instructions and time limit. Rating is based on a quantitative scoring system of a total of 18 different elements.

Tab. 1: Demographic profile of participants

	ALS	N	Amnestic MCI	N	Healthy controls	N	*p* value
	Mean±SD		Mean±SD		Mean±SD		
Age	60.4±12.2	40	64.6±5.4	39	62.7±10.2	40	0.152
Sex (male-female)	26-14	40	27-12	39	18-22	40	0.063
Education (years)	13.0±2.5	40	14.6±2.8	37	14.0±2.1	40	0.016
BDI-II	7.9±6.6	34	6.6±4.7	35	4.7±4.9	37	0.057
ALSFRS-R	38.4±7.8	40	na		na		na
Disease duration	22.2±19.5	40	na		na		na
Disease progression	0.5±0.4	40	na		na		na

2.2.3. Data analysis

Dependent demographic variables included age, education, and depression scores. Differences in gender distribution across groups were assessed using the Chi-Square Test. Differences in demographic and neuropsychological variables were assessed using one-way analysis of variance (ANOVA) with the main factor group (ALS/aMCI/HC). Neuropsychological variables included: letter fluency, letter flexibility, semantic fluency, semantic flexibility, cognitive flexibility, working memory, short term memory, learning, immediate recall, delayed recall, recognition, visuo-spatial skills. In case of missing data, cases were excluded from the individual analyses while retaining them in the dataset. The statistical threshold was set to $p < 0.05$. If applicable, post-hoc tests were conducted with the alpha level adjusted to $p < 0.017$ following Bonferroni correction. In order to determine whether the patient groups could be differentiated with less refined neuropsychological measures, composite scores for "Memory" and "Executive function" were computed for the study groups, reflecting temporal lobe and frontal lobe function, respectively. This was done by transforming the raw values into Z scores that were referenced by mean and standard deviation of the healthy control group in the corresponding test. All measures were adjusted such that a negative Z score indicates worse performance. Composite "Memory" scores included the following parameters: learning, immediate and delayed recall, recognition. The "Executive function" score included letter fluency and flexibility, working memory, and cognitive flexibility. Semantic fluency and flexibility were not considered as they are known to depend on temporal lobe mediated processes (Martin et al., 1994) and therefore likely to bias the "Executive function" score. Composite scores were compared across patient groups (ALS/aMCI) using t-tests for independent samples. In order to estimate the impact of executive function on memory performance, an additional regression analysis was performed within each group with "Memory" scores being used as the dependent variable and "Executive function" scores as the independent variable.

2.3. Results

2.3.1. Demographic characteristics

The groups did not differ in age ($F_{2,116} = 1.92$, $p = 0.152$), gender ($X^2 = 5.53$, $p = 0.063$), and self-reported depression scores ($F_{2,103} = 2.94$, $p = 0.057$). Although ALS patients tended to

report higher depression scores, none of the three groups scored within the range of clinically relevant symptoms (< 13 points). There was a difference between groups regarding their education ($F_{2,114} = 4.28$, $p = 0.016$). ALS patients completed less educational years than aMCI patients ($p = 0.013$) but both patient groups did not differ from healthy controls (Table 1).

The analysis of composite scores revealed no difference between the patient groups for "Memory" (t = 1.194, p = 0.236) and "Executive function" (t = -1.052, p = 0.296). The within-group regression showed no significant impact of "Executive function" on overall "Memory" performance for either the aMCI patients (R = 0.250, p = 0.125) or the healthy controls (R = 0.024, p = 0.881). In ALS patients, there was a significant relationship between these domains (R = 0.453, p = 0.003) with "Executive function" accounting for 20.5% of the variance in "Memory" performance. Figure 2 illustrates the percentage of aMCI and ALS patients that fell below -1.5 standard deviations of the healthy control mean in each neuropsychological subtest.

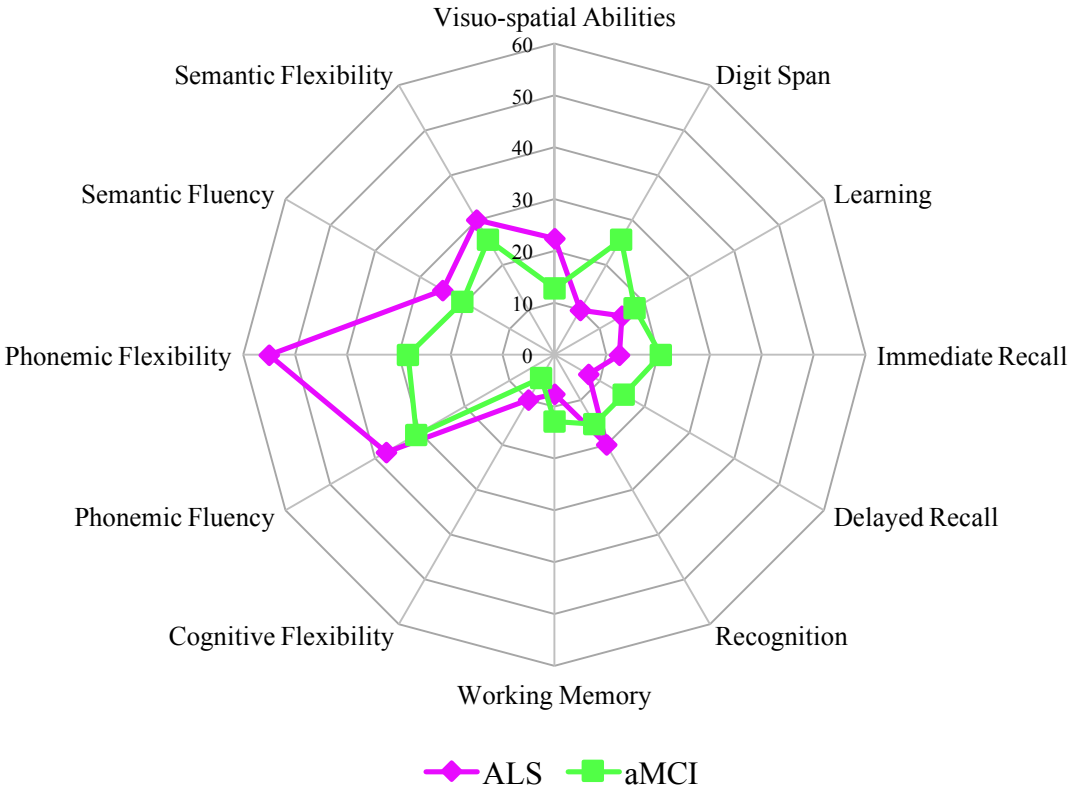

Fig. 2: Neuropsychological profile of patients with ALS (pink) and aMCI (green). Profile lines display the percentage of ALS patients and aMCI patients lying -1.5 SD below the mean of healthy controls for subtests within each cognitive domain.

2.3.2. Neuropsychological performance

Neuropsychological performance differed across groups in all cognitive domains (Figure 3). Test of executive functions revealed a significant effect of group in letter fluency ($F_{2,113}$ = 11.01, $p < 0.001$) and flexibility ($F_{2,112}$ = 18.47, $p < 0.001$), as well as in semantic fluency ($F_{2,112}$ = 6.24, $p = 0.003$) and flexibility ($F_{2,112}$ = 4.46, $p = 0.014$). Performance differences between groups were also observed in verbal working memory ($F_{2,114}$ = 10.48, $p < 0.001$), whereas this was not the case for cognitive flexibility ($F_{2,109}$ = 0.81, $p = 0.45$). In the tests of memory, a significant effect of group was identified for the digit span ($F_{2,114}$ = 8.58, $p < 0.001$), immediate ($F_{2,115}$ = 5.91, $p = 0.004$) and delayed ($F_{2,115}$ = 6.97, $p = 0.001$) verbal recall, and recognition ($F_{2,116}$ = 3.70, $p = 0.028$), while verbal learning did not differ between groups ($F_{2,116}$ = 2.67, $p = 0.074$). Visuo-spatial skills also significantly differed between ALS, aMCI, and controls ($F_{2,104}$ = 6.42, $p = 0.002$). Means, standard deviations, and post-hoc test results are summarized in Table 2.

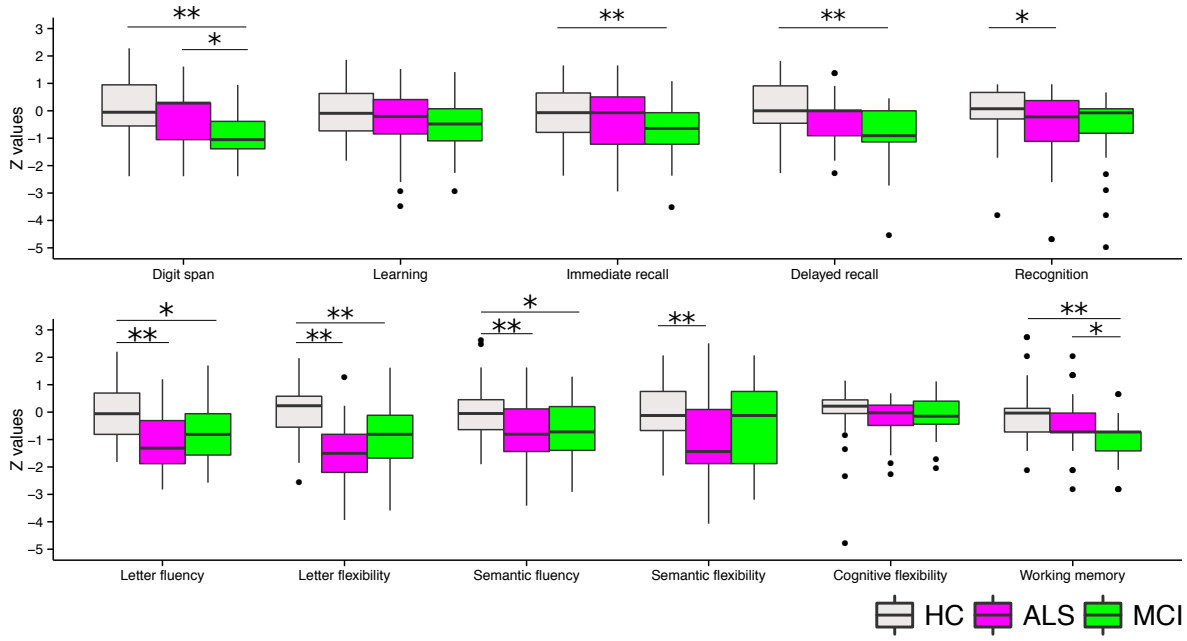

Fig. 3: Distribution of standardized neuropsychological performance in memory (top) and executive function (bottom). The boxes represent the median, first, and third quartile; the whiskers represent +/- 1.5* the interquartile range. * $p < 0.05$, ** $p < 0.01$

Tab. 2: Neuropsychological test results

	ALS	N	aMCI	N	HC	N	ALS vs HC	aMCI vs HC	ALS vs aMCI
							p value	*p* value	*p* value
Executive functions									
Letter fluency	10.9±4.5	40	12.4±4.1	36	15.2±4.0	40	**< 0.001**	**0.011**	0.395
Letter flexibility	9.0±3.3	40	10.7±3.5	35	13.3±2.9	40	**< 0.001**	**0.002**	0.079
Semantic fluency	20.5±6.5	40	21.7±6.3	35	25.3±6.0	40	**0.003**	**0.045**	1.000
Semantic flexibility	13.1±3.8	40	14.2±3.5	35	15.3±2.3	40	**0.010**	0.470	0.440
Working memory	5.5±1.5	38	4.6±1.2	39	6.1±1.5	40	0.260	< 0.001	**0.020**
Cognitive flexibility	2.8±1.0	34	2.6±1.0	38	2.5±1.4	40	-	-	-
Memory									
Digit span	7.2±1.5	38	6.3±1.3	39	7.6±1.5	40	0.900	**< 0.001**	**0.013**
Learning	44.3±10.5	40	42.6±8.2	39	47.3±9.0	40	-	-	-
Immediate recall	2.6±1.7	39	3.2±1.8	39	1.9±1.7	40	0.191	**0.003**	0.373
Delayed recall	2.7±1.8	40	3.7±2.2	39	2.0±2.2	39	0.411	**< 0.001**	0.082
Recognition	9.1±5.9	40	9.4±4.9	39	11.8±3.4	40	**0.042**	0.095	1.000
Visuo-spatial skills									
Rey figure	31.0±3.8	34	33.4±2.6	33	33.1±2.6	40	**0.010**	1.000	**0.005**

2.4. Summary

Study 1 revealed a significant deficit in verbal memory function in both ALS and aMCI patients in comparison to healthy controls. Importantly, the observed deficits in ALS were different from those observed in patients with Alzheimer's pathology, in that the ALS patients showed intact learning and recall but deficient recognition performance. Amnestic MCI patients, in turn, showed deficits in learning and recall but not in recognition performance. The overall memory performance in ALS was only explained to some extent by the coexisting executive dysfunction (20.5%), supporting the emerging notion that other cognitive domains,

such as memory, are affected in ALS pathology. These findings highlight the qualitative differences in temporal lobe dysfunction between ALS and aMCI patients, while suggesting temporal lobe dysfunction as a mechanism underlying the distinct cognitive impairments observed in ALS.

3. Study 2: Hippocampal volume and shape in ALS

3.1. Introduction

The results of Study 1 suggest that memory impairment in ALS is different from that observed in patients with Alzheimer's-related pathology, but the underlying anatomical correlates of these findings are unknown. Many brain regions have been implicated in the processing of memory, and it is the structures of the medial temporal lobe, namely the hippocampus, the parahippocampal gyrus and the entorhinal cortex, that play a crucial role in the formation of memory (Squire and Zola-Morgan, 1991). Specifically, the degeneration of the hippocampus has been related to memory deficits in Alzheimer's Disease (AD), even in early disease stages (Gosche et al., 2002; Mueller et al., 2010). In ALS, histopathological studies reported fibrillary gliosis and loss of myelinated fibers in the white matter in patients with concomitant dementia along the perforant pathway (Takeda et al., 2007), which was different from AD specific hippocampal lesions (Takeda et al., 2009). Although hippocampal volume reductions have been observed before *in vivo* (Abdulla et al., 2014; Bede et al., 2013c) and were related to patients' memory performance (Abdulla et al., 2014; Raaphorst et al., 2015), the exact location of these changes is still undetermined as volumetric measures only indicate a global volume loss. Surface-based approaches could complete the picture by revealing local shape alterations within the hippocampus, as it is a heterogeneous structure and, as such, it is likely that the pathology related to ALS may only occur in some portions of the hippocampus, rather than in its entirety. Specifically, the hippocampus can be subdivided into different cytoarchitectonic subfields encompassing the cornu ammonis fields CA1-4, the dentate gyrus (DG), and the subiculum, or, on a functional level, it can be separated into an anterior-posterior gradient along the longitudinal axes (Moser and Moser, 1998; Poppenk et al., 2013). To determine the precise patterns of morphologic degeneration within the various regions of the hippocampus in ALS, we investigated volumetric and shape differences using manual and automated hippocampal segmentation of T1-weighted structural magnetic resonance imaging (MRI) data. We hypothesized that previously reported volume reductions within the hippocampus in ALS are associated with local hippocampal subfield changes and can be identified using a surface-based approach.

3.2. Methods

3.2.1. Participants

Thirty-one patients with ALS were recruited from the outpatient clinic of the department of Neurology at the Otto-von-Guericke University. Patients were classified according to the revised El Escorial criteria (Brooks et al., 2000), and disease severity was rated using the ALS functional rating scale revised (ALSFRS-R, Cedarbaum et al., 1999). A group of 29 healthy controls without prior history of neurological or psychiatric illness served as a control group. Demographic characteristics of both groups are summarized in Table 3. All participants were different from those of Study 1. The local ethics committee of the Otto-von-Guericke University, in Magdeburg, Germany, approved the study and all participants gave written informed consent prior to their inclusion.

Tab. 3: Demographic profile of participants

	N	Age (years)	Sex (male – female)	Handedness (right – left)	ALSFRS-R	Site of onset (bulbar – spinal)	Disease duration (months)
ALS	31	62.8±13.0	20-11	28-3	37.8±5.4	8-23	21.6±21.0
HC	29	61.8±5.9	19-10	29-0	na	na	na
p Value		0.354	0.935	0.086	na	na	na

3.2.2. MRI acquisition

Three-dimensional, T1-weighted, structural MRI scans of the whole brain were acquired on a GE Signa Horizon LX 1.5T neuro-optimized magnetic resonance system (General Electric Co., Milwaukee, WI) using a standard quadrature head coil (contrast-optimized spoiled gradient-echo sequence, TE = 8 ms, TR = 24 ms; flip angle = 30°; voxel size = 1.0x1.0x1.5mm³).

3.2.3. Manual hippocampal volumetry and shape analysis

Prior to manual segmentation, T1-weighted images were resampled to 1mm isotropic voxels and registered into standard space using a 6 degrees of freedom (DOF) rigid body transformation to correct for variation in head tilt using FLIRT (Jenkinson et al., 2002), which is part of the FMRIB's Software Library (FSL) (Smith et al., 2013). Manual segmentation of the left and right hippocampi was conducted by one rater (who was blind to group allocation) using Multitracer (http://www.loni.usc.edu/Software/MultiTracer), which is a java-based tool for anatomic delineation of grayscale volumetric images (Woods, 2003). The software enables the simultaneous viewing of the hippocampus on three orthogonal planes. The border of the hippocampus was traced from rostral to caudal in magnified images of the coronal slices while simultaneously visualizing the sagittal orientation. Delineation was performed following standardized guidelines (Pruessner et al., 2000), using a freehand spline drawing technique that is considered to offer more precision than the previously used voxel-by-voxel approaches (Wisse et al., 2012). Segmentation included the hippocampus, the subiculum, and dentate gyrus, with white matter of the alveus and fimbria being excluded (Figure 4). The total volume of each hippocampus was calculated by summing the areas for each plane multiplied by the slice thickness. Each hippocampal volume consisted of about 35 to 45 individually segmented planes.

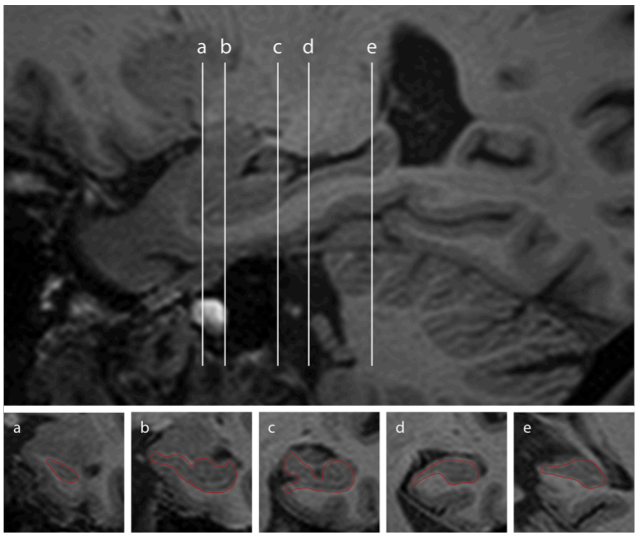

Fig. 4: Example of manual segmentation. The hippocampi were traced in contiguous coronal slices (rostral to caudal a-e). This figure is reproduced with permission from Abdulla et al. (2014).

Shape analysis was conducted using the freely-available shape tool software, developed by the laboratory of NeuroImaging (LONI), University of California, Los Angeles (http://www.loni.usc.edu/Software/ShapeTools). The digitized points derived from the manual segmentation representing the hippocampal contours in each brain slice were made spatially uniform by interpolation onto a parametric grid of 100x150 surface points describing the hippocampal surface of each subject (Narr et al., 2004). This procedure also enables the

generation of an average hippocampal surface model of all subjects, where statistical results can be mapped on. To assess between-group differences in hippocampal shape, a medial curve along the anterior-posterior axis was derived for each individual surface model (Narr et al., 2004). Subsequently, the radial distances from the hippocampal midline to the surface boundary were computed and the resulting vertices were used within a general linear model implemented in R (https://www.r-project.org/), with group as a main factor, and age and total intracranial volume (TIV) as covariates of no interest. TIV calculation is described in 3.2.5. The resulting *T* values for each vertex location and their corresponding *p* values were used to calculate the overall statistical significance of the radial shape differences between groups. Correction for multiple comparisons was achieved by permutation testing ($p < 0.05$) (Nichols and Holmes, 2002) using the statistical software R. The maximum cluster size was determined with a flood-fill algorithm, implemented in MATLAB (http://de.mathworks.com/products/matlab/).

3.2.4. Automatic hippocampal volumetry and shape analysis

Automatic segmentation of the left and right hippocampi was performed using FMRIB's Integrated Registration & Segmentation Tool (FIRST). FIRST incorporates prior anatomical information of 8 different subcortical structures separated for the left and right hemisphere via the usage of explicit shape models (Patenaude et al., 2011). These models were constructed from 336 manually segmented subjects with an age range from 4 to 87 years, and they include both normal and pathological brains (adults: schizophrenia, Alzheimer's disease, healthy controls; children: attention deficit disorder, prenatal cocaine exposure, schizophrenia, healthy controls, Patenaude et al., 2011). Prior to segmentation, T1-weighted raw images were skull stripped using the brain extraction tool BET (Smith, 2002), with the optional –B flag implemented to reduce image bias and residual neck voxels. Skull-stripped images were linearly registered to the MNI space (1mm MNI152 template) using 12 degrees of freedom (DOF), followed by a second stage registration to a MNI subcortical mask using FSL FLIRT (Jenkinson et al., 2002), which provides a more accurate and robust subcortical alignment (Patenaude et al., 2011). To obtain hippocampal volume and shape, FIRST uses a Bayesian probabilistic model that relies not only on average shape and intensity information from the training data set but also on modes of variation, that efficiently describe the ways in which the structure's shape varies most typically over a population. For each individual data set, the best shape is then determined by an iterative fitting of the model, which is described by meshes.

The volumetric output from the mesh is derived by identifying the voxels through which the mesh passes (boundary voxels) and filling the area within these voxels (Patenaude et al., 2011). Boundary correction was done using FAST (Smith, 2002) tissue classification to ensure that neighboring structures do not overlap.

In addition to the volumetric information, the individual meshes can be further used for shape analysis between groups. For that purpose, the vertex locations from each subject were projected onto the surface of an average template shape as scalar values, where a positive value is outside the surface and a negative is inside. Similar to the manual shape analysis, TIV and age were included as covariates of no interest, while group was used as the between-group factor within a general linear model. Intergroup differences were assessed at each vertex location using vertex-wise threshold-free cluster enhanced (TFCE) parameters (Smith and Nichols, 2009), which were permuted using FSL Randomise (Winkler et al., 2014). Results were corrected for multiple comparisons across space (FWE < 0.05).

3.2.5. Statistical analysis

Prior to statistical analysis, both manually and automatically extracted hippocampal volumes were adjusted for total intracranial volume (TIV) using the covariance method (Jack et al., 1989): adjusted hippocampal volume (HV) = original HV of each subject – α (TIV subject – mean TIV of the healthy controls), where α describes the slope of the regression between the HV and TIV in healthy controls. TIV was calculated in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) using the Gaussian mixture model within the unified segmentation approach (Ashburner and Friston, 2005), which entails the summation of the individual tissue classes (grey matter, white matter, cerebrospinal fluid), with a threshold of 0.5 (Pengas et al., 2009).

Demographic and volumetric data were plotted and visually inspected as well as tested for significant deviation from the normal distribution using Shapiro-Wilk test. Demographic data were not normally distributed and differences between groups were assessed using chi-square (gender, handedness) and Kruskal-Wallis (age) tests. Differences in hippocampal volume were assessed conducting one-way analysis of covariance (ANCOVA) with group as main factor (ALS/HC) and age as a covariate of no interest. Within the patient cohort, the clinical parameter disease severity (ALSFRS-R) was normally distributed, whereas disease duration

was not. In order to determine the relationship between hippocampal volume and these clinical parameters, Spearman rank correlations were computed with the significance level adjusted to $p = 0.025$ following Bonferroni correction.

3.3. Results

3.3.1. Hippocampal volume

Right hippocampal volumes were significantly reduced in ALS when adjusting for TIV and correcting for age (manual segmentation: $F = 4.61$, $p = 0.04$; automatic segmentation: $F = 7.04$, $p = 0.01$). The identified volume reduction was irrespective of the type of segmentation. The volumes of both the manually and the automatically segmented left hippocampi did not differ between groups (manual segmentation: $F = 3.15$, $p = 0.08$; automatic segmentation: $F = 3.05$, $p = 0.09$). Figure 5 displays the distribution of volumes derived from the manual and automatic segmentation, as well as the estimated marginal means adjusted for age. The mean manually segmented left hippocampal volumes were 2486 ± 370 mm^3 in ALS and 2656 ± 347 mm^3 in healthy controls; mean manually segmented right hippocampal volumes were 2600 ± 310 mm^3 in ALS and 2775 ± 316 mm^3 in healthy controls. Mean automatically segmented left hippocampal volumes were 3300 ± 507 mm^3 in ALS and 3501 ± 342 mm^3 in healthy controls; mean automatically segmented right hippocampal volumes were 3175 ± 483 mm^3 in ALS and 3492 ± 427 mm^3 in healthy controls.

Hippocampal volumes were not associated with patients' physical disability as measured with the ALSFRS-R (right manual segmentation: $\rho = -0.10$, $p = 0.59$; right automatic segmentation: $\rho = 0.04$, $p = 0.82$; left manual segmentation: $\rho = -0.06$, $p = 0.76$; left automatic segmentation: $\rho = 0.10$, $p = 0.61$) and disease duration (right manual segmentation: $\rho = -0.2$, $p = 0.28$; right automatic segmentation: $\rho = -0.31$, $p = 0.09$; left manual segmentation: $\rho = -0.27$, $p = 0.14$; left automatic segmentation: $\rho = -0.25$, $p = 0.17$).

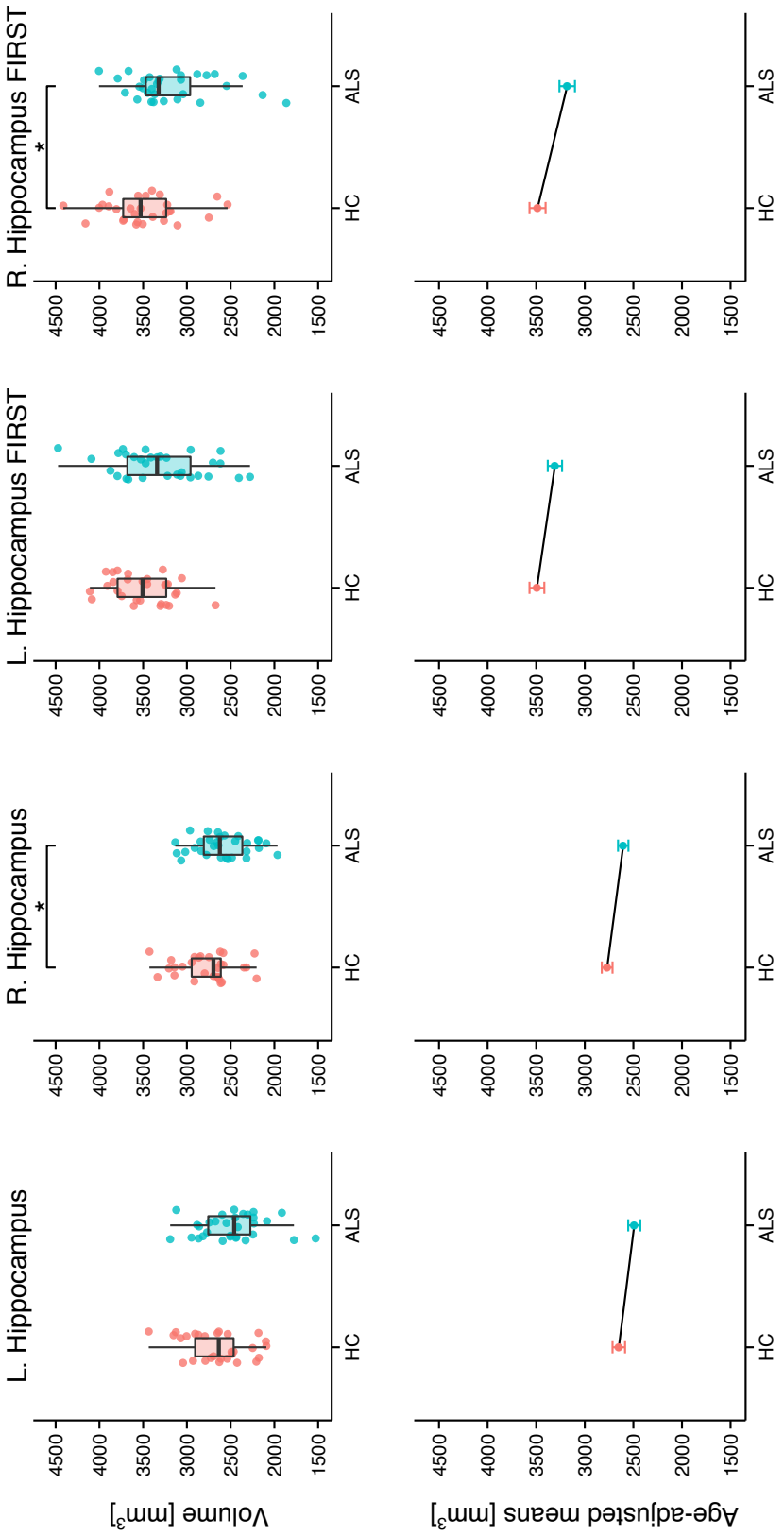

Fig. 5: Hippocampal volumetric differences between patients with ALS and healthy controls. Boxplots display the distribution of hippocampal volumes, derived from manual (left) and automatic (right) segmentation and the age-adjusted marginal means. FIRST: FMRIB's Integrated Registration & Segmentation Tool, * $p < 0.05$

3.3.2. Hippocampal shape

Permutation tests of vertices describing the hippocampal shape derived from manual segmentation revealed no significant difference in clustersize between healthy controls and ALS patients for both the left (clustersize = 295, $p = 0.148$) and the right (clustersize = 106, $p = 0.605$) hippocampus. Based on the proposed functional specialization along the longitudinal axes (Poppenk et al., 2013), region-of-interest (ROI) analyses were conducted for the hippocampal head, body, and tail. Permutation testing revealed local shape differences between the groups in the left (clustersize = 295, $p = 0.049$), but not for the right hippocampal head (clustersize = 106, $p = 0.282$) (Figure 6). No significant clusters were found in the bilateral hippocampal body and tail.

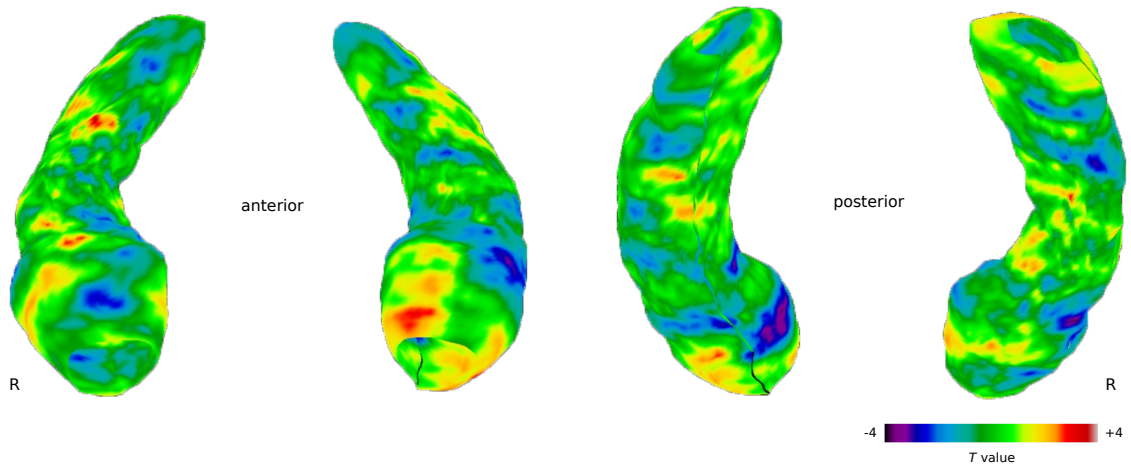

Fig. 6: Manual hippocampal shape analysis. The color bar indicates differences in radial distances between groups obtained at each hippocampal surface collection. Negative *T* values index surface shrinkage, positive *T* values index surface coves in ALS compared to healthy controls. The cluster in the left hippocampal head is significant after correction for multiple comparisons ($p < 0.05$).

Automated hippocampal vertex-wise analysis revealed shape deformities in the right hippocampal head and body in ALS in comparison with healthy controls (Figure 7) following FWE correction ($p < 0.05$). For the left hippocampal formation, no shape deformities were detected in the ALS patients in comparison to the healthy controls.

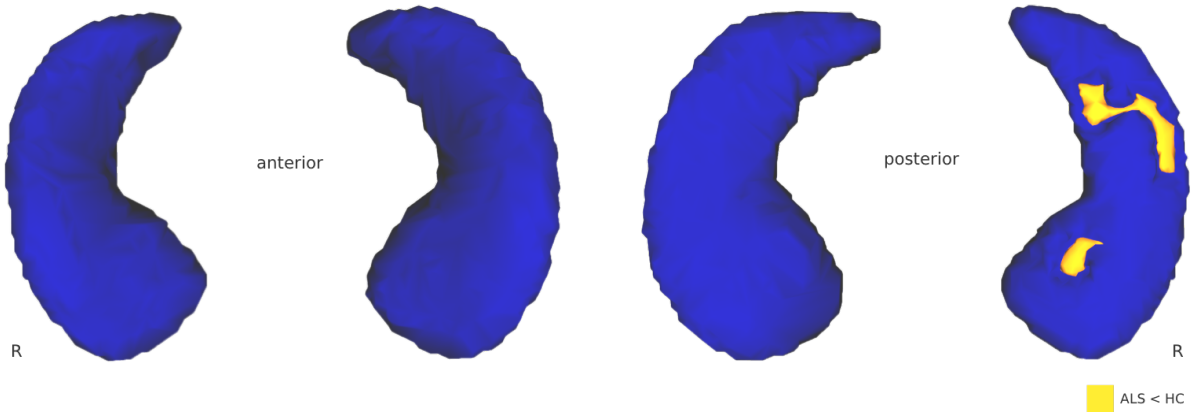

Fig. 7: Automated hippocampal shape analysis. Blue color indicates the 3-dimensional template mesh, orange highlights ALS-related local shape deformations (ALS < HC). Results are corrected for multiple comparisons across space (FWE < 0.05).

3.4. Summary

Study 2 investigated the patterns of hippocampal degeneration in ALS using a volumetric and surface-based approach. The results provide evidence for ALS-related structural alterations in the hippocampal formation that are characterized by global volume loss and local shape deformation in the anterior part of the hippocampus. Importantly, these differences were present irrespective of the type of segmentation (manual vs. automatic). Hippocampal volume loss in ALS was detected in both left and right hippocampi, although volume reductions in the left hippocampus did not reach the predetermined significance level of $p < 0.05$. Local shape alterations were identified in the hippocampal head region that corresponds to the cornu ammonis field 1 (CA1), a region known to be involved in novelty detection, memory processing and integration of hippocampal input and output information. Taken together, the results of Study 2 suggest a global hippocampal volume loss in ALS that is complemented by local shape deformations in a region involved in the integration of input and output information. In combination with Study 1, these findings support the view of the presence of a substantial temporal lobe deficit, which is associated with the disease pathology.

4. Study 3: Subcortical pathology in ALS is associated with neuropsychological deficits[2]

4.1. Introduction

The results from Studies 1 and 2 demonstrate that memory deficits in ALS are different from a pure amnestic syndrome and could be related to a structural lesion in the hippocampal CA1 region. Apart from memory deficits, other neuropsychological deficits are reported frequently in ALS such as behavioral deficits (Lillo et al., 2011), executive dysfunction (Phukan et al., 2007), and more recently, language deficits (Abrahams, 2013). The distinct neuropsychological profile of ALS points beyond a focal cortical pathology and suggests a complex frontostriatal dysfunction. Frontostriatal neural loops consist of parallel circuits with discrete neurobehavioral functions (Bonelli and Cummings, 2007) and impairments of these circuits manifest as network-specific neuropsychological deficits.

Dysfunction of the dorsolateral prefrontal-subcortical circuit is associated with executive dysfunction, whereas disruption of the lateral orbitofrontal-subcortical circuit has been associated with disinhibition and impulsivity (Mega and Cummings, 1994). The anterior cingulate-subcortical circuit mediates motivation, and changes in the integrity of this circuit are thought to manifest in apathy (O'Callaghan et al., 2014). All of these networks are relayed through the thalamus and pallidum, where ALS-related pathology has been described in a histopathology case series (Brownell et al., 1970). Further studies described subcortical volume loss in the putamen, caudate nucleus (Agosta et al., 2009), hippocampus (Bede et al., 2013c), and the amygdala (Pinkhardt et al., 2006). Although all of those structures are part of cortico-basal neural loops (Alexander et al., 1986; O'Callaghan et al., 2014), previous imaging studies have not related subcortical pathology to cognition and behavior. As Studies 1 and 2 focused specifically on the hippocampus and its contribution to the patients' cognitive profile, we endeavored here to characterize subcortical changes across the cognitive spectrum of ALS in a range of subcortical structures, hypothesizing that a continuum of incremental pathology

[2] The chapter is partially based on an article by Machts, J., Loewe, K., Kaufmann, J., Jakubiczka, S., Abdulla, S., Petri, S., Dengler, R., Heinze, H.J., Vielhaber, S., Schoenfeld, M.A., Bede, P. 2015. Basal ganglia pathology in ALS is associated with neuropsychological deficits. Neurology. doi:10.1212/WNL.0000000000002017.

can be captured *in vivo*. Additionally, we hypothesized that an ALS-specific pattern of subnuclear pathology may be identified within the basal ganglia.

4.2. Methods

4.2.1. Participants

A total of 67 patients with ALS and 39 healthy controls, partly overlapping with participants from Study 1, were included in this cross-sectional neuroimaging study. Patients were recruited from the Hannover Medical School and the Otto-von-Guericke University Magdeburg, Germany, over a period of three years. Patients were diagnosed according to the revised El Escorial Criteria (Brooks et al., 2000), and physical disability was rated using the revised ALS functional rating scale (ALSFRS-R) (Cedarbaum et al., 1999). Seven ALS patients fulfilled the diagnostic criteria for behavioral variant frontotemporal dementia (bvFTD) (Rascovsky et al., 2011). The remainder of patients was further categorized into patients with and without neuropsychological impairment using the Strong criteria (Strong et al., 2009)(see chapter 1.4.6), and allocated to the groups of “ALS-Plus” and “ALS-Nci”, respectively. A detailed description of classification criteria is given in the next section 4.2.2. Basic clinical and demographic data are summarized in Table 4. In order to maximize clinical homogeneity, patients with progressive muscular atrophy, primary lateral sclerosis, or flail limb phenotypes were excluded. All patients were screened for the presence of the GGGGCC hexanucleotide repeat expansion in *C9orf72* using repeat-primed polymerase chain reaction. A repeat length greater than 30 was considered positive for the expansion which was further verified by southern blotting. Given the strong imaging signature of the *C9orf72* repeat expansions (Bede et al., 2013b; Walhout et al., 2015), mutation carriers were not included in the analyses. Additional exclusion criteria for all participants included cerebrovascular disease, traumatic brain injury, and other neurological or psychiatric conditions. Healthy controls had to score within the normal range of the Montreal Cognitive Assessment (MoCA; (Nasreddine et al., 2005) to be included in the study.

Tab. 4: Demographic profile of participants

	N	Age (years)	Education	Sex (male – female)	Handedness (right – left)	ALSFRS-R	Disease duration (months)
HC	39	59.6±10.1	13.9±1.8	27-12	38-1	na	na
ALS-Nci	42	58.4±9.5	13.7±2.9	25-17	42-0	35.2±8.3	26.6±15.8
ALS-Plus	18	63.6±12.1	12.3±2.2	12-6	16-2	35.2±6.3	24.7±19.3
ALS-FTD	7	63.0±11.1	13.6±1.9	5-2	6-1	39.4±3.0	32.9±42.0
p Value		0.284	0.105	0.799	0.086	0.370	0.669

4.2.2. Neuropsychological assessment

All participants underwent a detailed neuropsychological assessment within two days of their brain scan, testing the domains of executive function, verbal memory, language, behavior, and visuo-spatial skills. For details on the neuropsychological test battery see 2.2.2. Performance on the following tests was utilized for neuropsychological classification: letter fluency and flexibility (Aschenbrenner et al., 2000), semantic fluency and flexibility (Aschenbrenner et al., 2000), trail making test (ratio between part B and A, Reitan, 1992), Stroop test (ratio between naming and reading; naming errors, Stroop, 1935), backward digit span (Härting et al., 2000), and the Frontal Systems Behaviour Scale (FrSBe). Patients were classified as ALS with cognitive impairment ("ALSci") if their performance was two standard deviations below the mean of healthy controls in at least two independent executive tests (Strong et al., 2009). The diagnosis of ALS with behavioral impairment (ALSbi) was established based on impairment in one of the subscales of the FrSBe, indicating apathy, disinhibition, or executive dysfunction. Questionnaires were filled in by the caregiver. Only those items that indicated a change in behavior after disease onset were considered for classification. Ten patients with "ALSci" and eight patients with "ALSbi" were merged to form the group "ALS-Plus". To account for motor impairment, fluency and flexibility indices were computed as suggested by Abrahams and colleagues, where fluency index (*fi*) = (60 seconds – time needed for reproducing words verbally or in writing) / total number of items generated (Abrahams et al., 2000). Cut-off values for each test and study group specific performance data are reported in Table 5.

Tab. 5: Group-specific neuropsychological performance.

	Cut-off	Nr. of impaired patients	ALS-Nci (Mean±SD)	N	ALS-Plus (Mean±SD)	N	ALS-FTD (Mean±SD)	N	F value	p value ALS-Nci vs. ALS-Plus	p value ALS-Nci vs. ALS-FTD	p value ALS-Plus vs. ALS-FTD
Letter fluency	6.7	8	4.1±1.9	36	6.8±2.5	14	7.5±1.8	4	12.1	<.001	0.007	ns
Letter flexibility	7.3	10	4.6±1.5	34	8.9±6.9	14	8.8±2.0	4	7.2	0.002	ns	ns
Semantic fluency	3.5	3	1.9±0.5	34	2.8±1.1	14	3.8±1.1	4	17.1	0.001	<.001	0.055
Semantic flexibility	5.1	5	3.2±0.8	34	4.6±1.2	14	6.3±2.2	4	7.1	<.001	<.001	0.024
Stroop ratio	1.5	2	1.1±0.1	35	1.5±0.6	12	1.3±0.3	5	ns	-	-	-
Stroop errors	2.4	11	1.6±3.9	35	2.3±2.0	12	6.0±10.3	5	ns	-	-	-
Cognitive flexibility	3.9	8	2.3±1.0	41	3.0±1.7	18	3.6±0.9	6	4.2	ns	0.052	ns
Digit span backwards	3.1	6	6.0±1.7	40	4.9±1.3	16	4.3±0.5	4	5.0	0.039	ns	ns
FrSBe Apathy	36.2	9	25.8±7.6	25	39.2±7.1	10	42.7±11.9	3	14.4	<.001	0.003	ns
FrSBe Disinhibiton	35.1	2	23.2±5.8	25	30.0±4.4	10	30.9±10.4	3	6.1	0.012	ns	ns
FrSBe Executive Function	55.7	0	31.4±8.7	25	39.4±8.3	10	50.4±5.4	3	8.7	0.047	0.013	ns
VLMT Learning	na	na	51.1±8.7	34	40.5±11.8	11	33.3±14.5	3	8.2	0.010	0.002	ns
VLMT Immediate recall	na	na	10.9±2.4	34	7.2±4.0	11	2.7±3.8	3	9.2	0.002	<.001	ns
VLMT Delayed recall	na	na	10.7±2.4	34	7.2±4.2	11	6.0±5.6	3	ns	-	-	-
VLMT Recognition	na	na	11.8±3.0	34	7.6±5.8	11	3.3±12.9	3	ns	-	-	-

4.2.3. MRI data acquisition

T1-weighted structural MRI scans were acquired on a 3T Siemens Magnetom VERIO scanner with a 32-channel head coil using a 3D-MPRAGE sequence (echo time (TE) = 4.82ms, repetition time (TR) = 2500ms, inversion time (TI) = 1100ms, flip angle = 7°, isotropic voxel size = 1mm³). To rule out confounding pathological findings, T2-weighted (gradient echo sequence: TE = 19.9ms, TR = 620ms, flip angle = 20°, voxel size = 1.1x0.9x5.0 mm³) and FLAIR (turbo spin-echo sequence: TE = 94.0ms, TR = 9000ms, TI = 2500ms, flip angle = 150°, voxel size = 1.0x0.9x5.0 mm³) sequences were also acquired and individually inspected.

4.2.4. Volumetric analyses

The volumes of seven subcortical structures were estimated separately for each hemisphere using the segmentation and registration tool FIRST, which is part of the FMRIB´s Software Library (FSL). These structures included the caudate nucleus, thalamus, nucleus accumbens, hippocampus, amygdala, putamen, and pallidum. Brain-extracted T1-weighted images were used for registration and subcortical segmentation (Figure 8) that was carried out as previously described in section 3.2.5. The resulting subcortical volumes were adjusted for total intracranial volume (TIV) using the covariance method (Jack et al., 1989). Image segmentation for TIV calculation was carried out based on a hidden Markov random field model using an expectation-maximization algorithm as implemented in the FSL tool FAST. TIV was calculated as the sum of partial volume estimates of the three main tissue components. To check for the asymmetry of the structural changes, an asymmetry index (AI) was calculated for each subcortical structure that did not differ between the study groups (Table 7). Accordingly, the pairwise sum of the left and right nuclei was used for each structure in subsequent analyses. A one-way analysis of covariance (ANCOVA) was conducted to explore intergroup differences in subcortical volumes using patient categorization as independent variable and age as a covariate of no interest. Post-hoc comparisons between groups were adjusted for multiple comparisons using Bonferroni correction. The significance threshold was set to 0.05. Additionally, a linear discriminant analysis was performed to evaluate the predictive value of subcortical volumes for phenotypic classification. The volumes of all subcortical structures were included in a stepwise analysis where at each step the predictor with the largest F value that exceeds the entry criteria

(F = 3.84) is added to the model (removal criteria: F = 2.71). The resulting model was cross-validated using leave-one-out classification. The overall *a priori* group prediction is 47.6%.

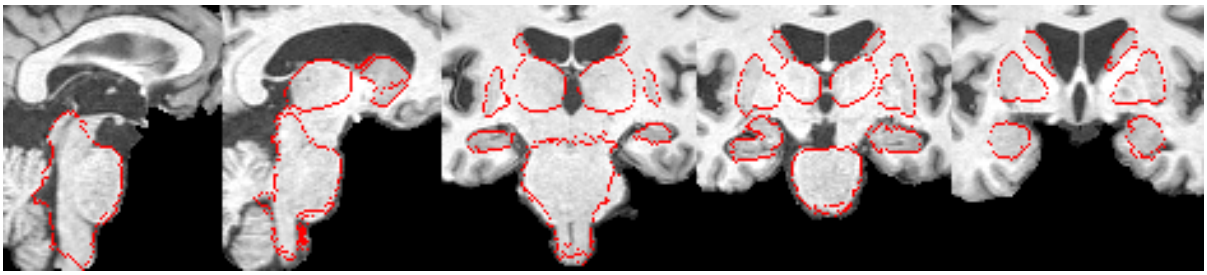

Fig. 8: Subcortical gray matter segmentation and registration example using FSL FIRST.

In order to rule out a confounding relationship between subcortical volumes and disease duration or patients' physical disability (ALSFRS-R), Pearson correlations were calculated within each patient group. In addition, the relationship between subcortical volumes and anatomically linked neuropsychological performance was explored, i.e. memory performance with hippocampal volumes, and apathy scores with nucleus accumbens volumes.

4.2.5. Shape analyses

Vertex-wise statistics for each structure were performed using the subcortical segmentation outputs generated by FIRST, as previously described in section 3.2.4. The vertex locations from each subject were projected onto the surface of an average template shape as scalar values, where a positive value is outside the surface and a negative is inside. TIV and age were included as covariates of no interest. Intergroup differences were assessed using permutation-based non-parametric testing as implemented in FSL "Randomise" (Winkler et al., 2014). Vertex-wise threshold-free cluster enhanced (TFCE) parameters (Smith and Nichols, 2009) were estimated and permuted to account for age and TIV. Results were corrected for multiple comparisons across space (FWE < 0.05).

4.2.6. Density analyses

In order to comprehensively characterize the extent and nature of subcortical involvement in ALS, we performed additional density analyses using FSL-VBM. Brain-extracted T1-weighted images of all participants were tissue-type segmented and a study-specific template

was created, consisting of seven brain scans of each group that did not differ in age, gender, education, and handedness. Gray matter partial volume estimates of all participants were non-linearly registered to the study-specific template, modulated by a Jacobian field warp to compensate for registration bias, and smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. A supplementary region-of-interest (ROI) gray matter density analysis was also performed within a combined basal ganglia mask, which included the bilateral caudate nuclei, thalami, accumbens nuclei, hippocampi, amygdalae, putamina, and pallida (Figure 9). To explore focal gray matter differences between groups (using age as a covariate), permutation-based non-parametric testing was applied using "Randomise", as implemented in FSL. Briefly, TFCE parameters were estimated for each voxel and permuted using the Freedman-Lane procedure to account for age (Freedman and Lane, 1983). This approach was used for both the whole-brain and ROI analyses. Results were corrected for multiple comparisons across space (FWE < 0.05).

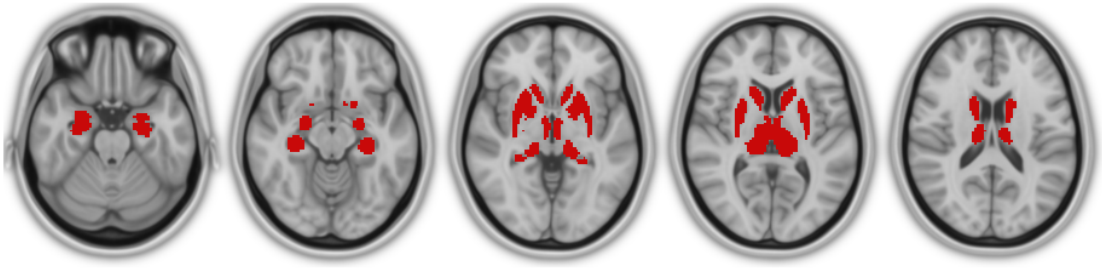

Fig. 9: Basal ganglia region-of-interest (ROI) mask. The mask includes the bilateral caudate nuclei, thalami, accumbens nuclei, hippocampi, amygdalae, putamina, and pallida and was created based on the Harvard-Oxford subcortical atlas.

4.3. Results

4.3.1. Volumes

Six subcortical structures showed intergroup differences when adjusting for TIV and controlling for age: the caudate nucleus ($p = 0.023$), thalamus ($p < .001$), nucleus accumbens ($p < .001$), hippocampus ($p < .001$), putamen ($p < .001$), and the pallidum ($p = 0.033$). No between-group differences were observed in the amygdala ($p = 0.406$). ALS-Plus patients exhibited significant hippocampal volume reductions compared to controls ($p = 0.025$) and ALS-Nci patients ($p = 0.052$), although the latter comparison did not reach the significance

level of 0.05. ALS-FTD patients showed significant volume loss in all of the subcortical structures with the exception of the amygdala compared to all other study groups. The means and standard deviations of subcortical volumes, asymmetry indices, and post-hoc test results are summarized in Table 7. Group-specific volume distributions for all structures are illustrated in Figure 10.

The stepwise discriminant analysis identified two statistically significant functions when including hippocampal and thalamic volumes (Wilks' Λ statistic for function 1 through 2 functions: $X^2 = 35.384$, $p < .001$; after removal of function 1: $X^2 = 4.889$, $p = 0.027$). Canonical correlations are displayed in Table 7 (Ld1 and Ld2). The overall accuracy of the model is 70.1% using one-leave out classification (Table 6).

Tab. 6: Confusion matrix of actual group membership

		Predicted (%)			
		ALS-Nci	**ALS-Plus**	**ALS-FTD**	**Total**
Actual	**ALS-Nci**	38 (90.5)	1 (2.4)	3 (7.1)	42
	ALS-Plus	13 (72.2)	5 (27.8)	0 (0)	18
	ALS-FTD	2 (28.6)	1 (14.3)	4 (57.1)	7

Patients' verbal memory performance correlated with hippocampal volumes (Figure 11): Learning ($r = 0.439$, $p = 0.002$), immediate recall ($r = 0.435$, $p = 0.002$), delayed recall ($r = 0.533$, $p < .001$), and recognition ($r = 0.448$, $p = 0.001$). A negative correlation was also identified between apathy scores and accumbens nuclei volumes, although it did not reach the significance level of 0.05 ($r = -0.318$, $p = 0.052$). No relationship was identified between subcortical volumes, disease severity (ALSFRS-R), and disease duration.

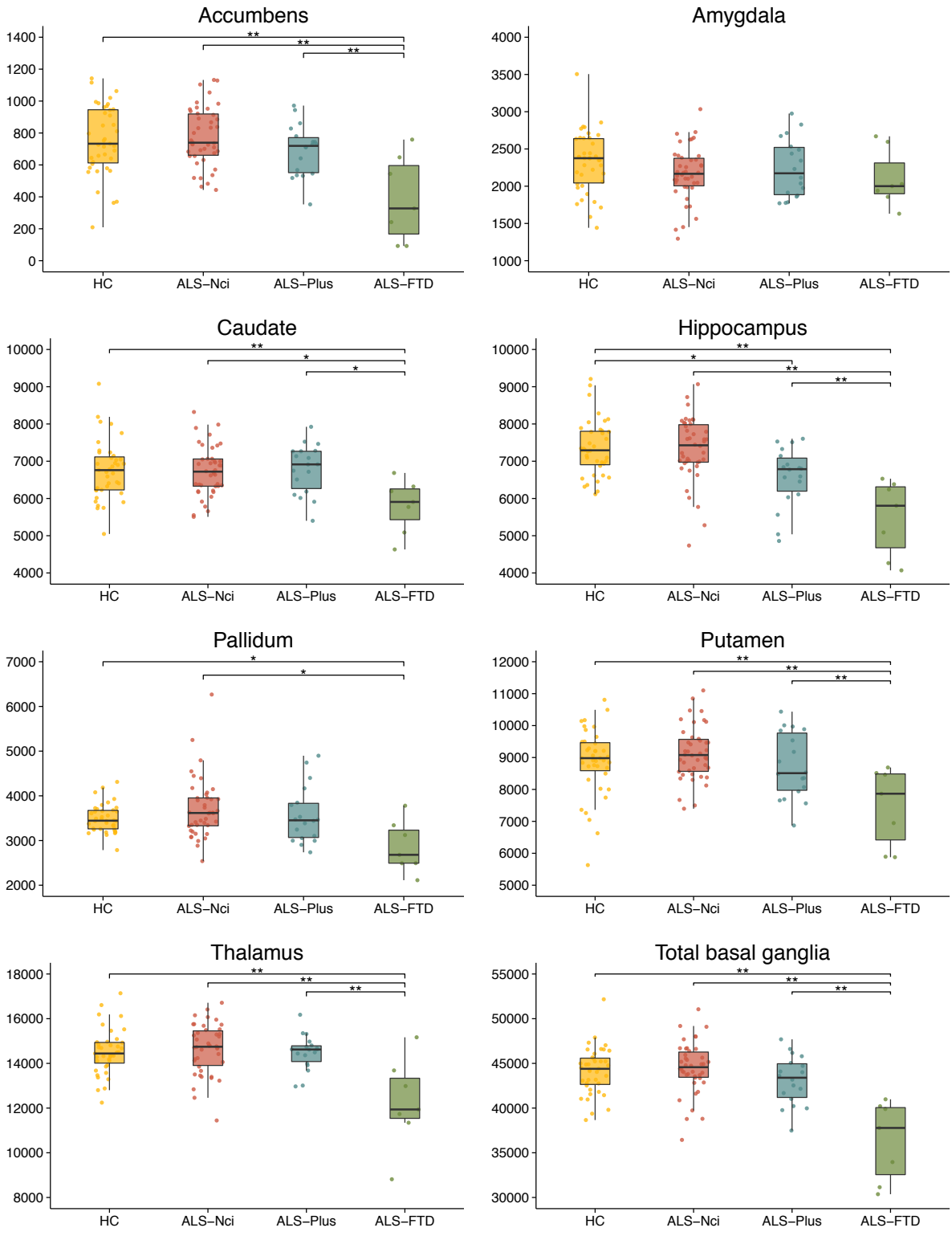

Fig. 10: Basal ganglia volumetric differences between the cognitive phenotypes of ALS and healthy controls. *$p < 0.05$, ** $p < 0.001$

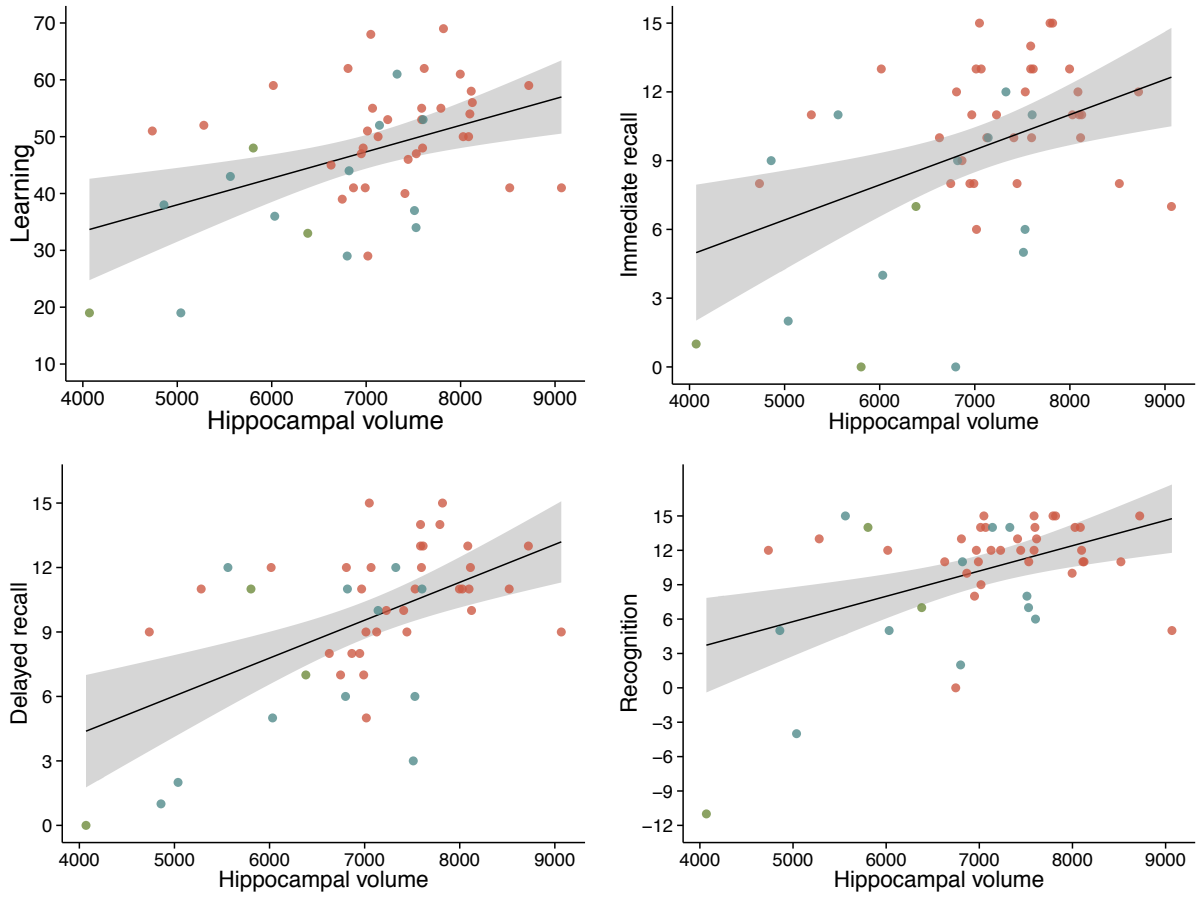

Fig. 11: Correlation between verbal memory performance and hippocampal volume of patients with ALS. Scatterplots show the association between total hippocampal volumes in ALS and verbal learning (top left), immediate recall (top right), delayed recall (bottom left) and recognition (bottom right) performance.

Tab. 7: Subcortical volumes.

Structure	Group	Mean AI	Volume (Mean±SD)	Estimated marginal means	Std. Error	Ld1	Ld2	F value	p value	Post hoc comparisons
Thalamus	HC	-0.01	14516	14501	171	0.777	0.629	10.34	< .001	HC vs. ALS-FTD p < .001 ALS-Nci vs. ALS-FTD p < .001 ALS-Plus vs. ALS-FTD p < .001
	ALS-Nci	-0.01	14643	14592	165					
	ALS-Plus	-0.03	14487	14602	254					
	ALS-FTD	-0.02	12239	12335	404					
Caudate	HC	0.05	6759	6758	115	0.163	0.035	2.96	0.023	HC vs. ALS-FTD p = 0.010 ALS-Nci vs. ALS-FTD p = 0.013 ALS-Plus vs. ALS-FTD p = 0.014
	ALS-Nci	0.03	6732	6729	111					
	ALS-Plus	0.04	6797	6806	171					
	ALS-FTD	0.07	5799	5806	272					
Putamen	HC	0.03	8858	8837	140	0.438	0.041	12.78	< .001	HC vs. ALS-FTD p = 0.005 ALS-Nci vs. ALS-FTD p = 0.001 ALS-Plus vs. ALS-FTD p = 0.007
	ALS-Nci	0.03	9135	9063	136					
	ALS-Plus	0.03	8736	8896	208					
	ALS-FTD	0.04	7464	7598	331					
Pallidum	HC	0.01	3601	3604	108	0.349	0.131	2.74	0.033	HC vs. ALS-FTD p = 0.042 ALS-Nci vs. ALS-FTD p = 0.011
	ALS-Nci	-0.01	3712	3725	105					
	ALS-Plus	-0.02	3565	3538	162					
	ALS-FTD	0.00	2860	2837	256					
Hippocampus	HC	0.01	7350	7336	123	0.872	-0.489	16.09	< .001	HC vs. ALS-Plus p = 0.025 HC vs. ALS-FTD p < .001 ALS-Nci vs. ALS-FTD p < .001 ALS-Plus vs. ALS-FTD p = 0.009
	ALS-Nci	0.00	7325	7276	119					
	ALS-Plus	-0.02	6582	6690	182					
	ALS-FTD	-0.02	5483	5574	290					
Accumbens	HC	-0.18	754	749	27	0.516	0.188	18.26	< .001	HC vs. ALS-FTD p <.001 ALS-Nci vs. ALS-FTD p <.001 ALS-Plus vs. ALS-FTD p = 0.001
	ALS-Nci	-0.20	777	760	26					
	ALS-Plus	-0.18	691	728	41					
	ALS-FTD	-0.27	386	417	65					
Amygdala	HC	0.03	2321	2321	62	0.171	-0.017	1.01	0.406	
	ALS-Nci	0.03	2166	2166	60					
	ALS-Plus	0.00	2241	2240	93					
	ALS-FTD	-0.01	2103	2103	148					

4.3.2. Shape analyses

Vertex-wise analyses revealed right ventral amygdala atrophy in ALS-Nci patients and bilateral hippocampal atrophy in ALS-Plus in comparison to controls (Figure 12). ALS-FTD patients showed extensive vertex-wise changes in the bilateral thalami, caudate nuclei, putamina, pallida, hippocampi, and accumbens nuclei compared to all other groups (Figure 12), revealing specific sub-regions of vulnerability within those structures. Thalamic shape analyses indicated pathological changes in the anterior nuclei, the ventral anterior nucleus, the lateral dorsal nuclei, and the internal medullary lamina. Caudate pathology was localized to the bilateral heads and right tail of the structure. Putamen atrophy was predominantly confined to the dorsal portion of the structure. Both the nucleus accumbens and the pallidum were considerably affected in a concentric pattern. Hippocampal changes involved the head and body of the structure. While the anatomical pattern was similar, surface-projected changes were least pronounced in ALS-Nci, more severe in ALS-Plus, and most marked in ALS-FTD.

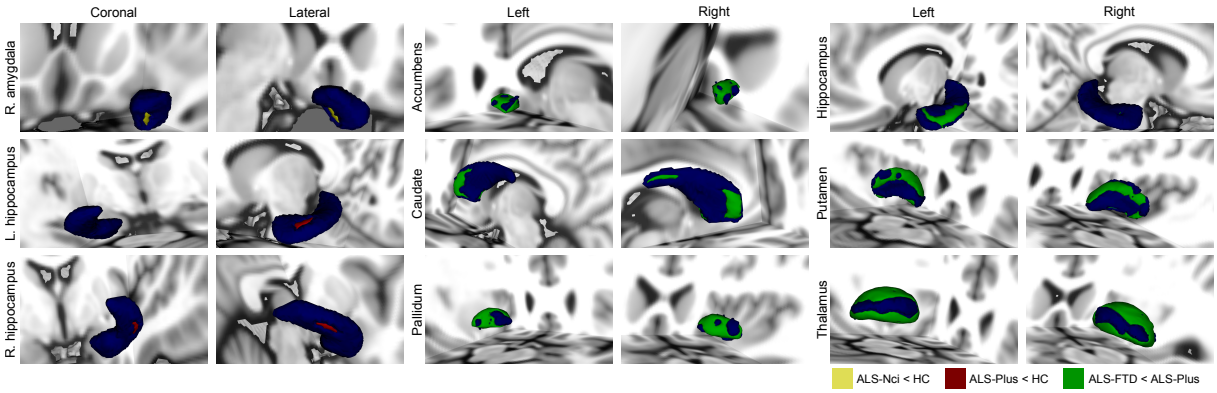

Fig. 12: Subcortical shape differences between disease groups and healthy controls. Blue color indicates the 3-dimensional template mesh of the given structure. Yellow color highlights the affected aspect of the structure in ALS-Nci compared with healthy controls (left), red color highlights the affected regions in ALS-Plus compared with healthy controls (left), and green color indicates shape differences in ALS-FTD compared with ALS-Plus (middle, right). Results are corrected for age and total intracranial volume (FWE < 0.05).

4.3.3. Density analyses

Whole-brain VBM revealed gray matter density reductions in the right precentral gyrus, right middle frontal gyrus, and cerebellum in ALS-Nci patients compared to controls (Figure 13a). Consistent with the volumetric and shape findings, hippocampal density reduction was

observed in ALS-Plus patients, albeit at a lower statistical threshold ($p < .001$, uncorrected). Finally, widespread gray matter atrophy was identified in the ALS-FTD group compared to all other groups, affecting the frontal and temporal lobes, the cerebellum, and the basal ganglia. While the anatomical pattern was identical, the extent of atrophy observed in ALS-FTD was more marked in comparison to ALS-Nci than when it was compared to ALS-Plus (Figure 13b). The pattern of incremental gray matter pathology along the cognitive continuum of ALS was further supported by the ROI density analysis (Figure 13c).

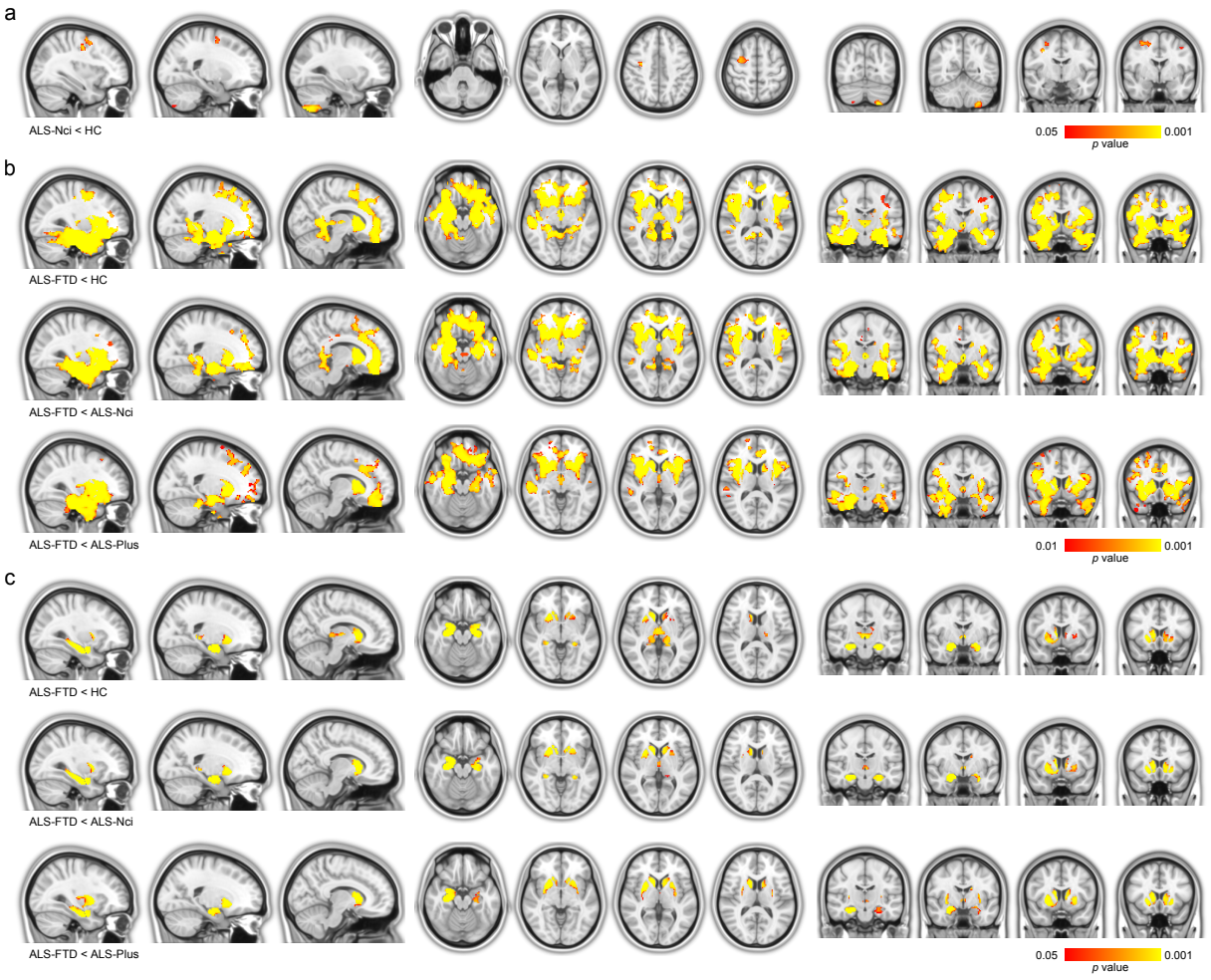

Fig. 13: Patterns of reduced gray matter density in (a) ALS-Nci compared with healthy controls; (b) ALS-FTD compared with healthy controls (top), ALS-Nci (middle), and ALS-FTD (bottom). (c) Clusters of reduced gray matter density within a combined basal ganglia mask in ALS-FTD compared with healthy controls (top), ALS-Nci (middle), and ALS-FTD (bottom). Results are corrected for age (FWE < 0.05). Images follow radiologic convention.

4.4. Summary

The results from Study 3 provide evidence for an incremental gradient of subcortical pathology across the ALS – ALS-FTD spectrum, suggesting that the degree of subcortical gray matter pathology in ALS is closely associated with neuropsychological changes. Complementary to Studies 1 and 2, the present results show hippocampal pathology associated with the patients' cognitive status, and indicate that hippocampal and thalamic volumes discriminate best between ALS cognitive subgroups. The combination of the results from Studies 1-3 indicate a network-specific degeneration of cortical-subcortical networks that modulates the unique neuropsychological profile of ALS.

5. Study 4: Functional connectivity changes in ALS indicate widespread temporal lobe pathology[3]

5.1. Introduction

As demonstrated in Studies 1-3, there is strong evidence of temporal lobe involvement in ALS at both the behavioral and the structural level. By contrast, recent investigations of functional alterations in extra-motor areas are rather inconclusive, particularly with regard to the idea of a clinical continuum with FTD.

ALS-related structural changes, such as cortical thinning (Schuster et al., 2013; Walhout et al., 2014), decreased connectivity (Douaud et al., 2011; Verstraete et al., 2011), and grey matter atrophy (Grosskreutz et al., 2006) have been consistently found for the motor cortex. A similar pattern of degeneration, although less consistent, was reported for functional changes within the motor system with heightened bilateral activation of motor areas (Schoenfeld et al., 2005) and functional recruitment of adjacent regions (Konrad et al., 2002) during task fMRI, as well as decreases in motor functional connectivity at rest (Fekete et al., 2013; Jelsone-Swain et al., 2010; Mohammadi et al., 2009).

In contrast to the well-described motor degeneration, the evidence from neuroimaging studies for extra-motor involvement in ALS is rather heterogeneous. With respect to the continuum hypothesis, a gradient of cortical (Mioshi et al., 2013) and subcortical (Machts et al., 2015) grey matter pathology along the cognitive phenotypes of ALS has been observed in the frontal and temporal lobes using structural imaging, while studies using resting state fMRI reported either increased (Agosta et al., 2013a; Agosta et al., 2011; Douaud et al., 2011) or decreased (Agosta et al., 2013a; Luo et al., 2012; Mohammadi et al., 2009) extra-motor functional connectivity. This heterogeneity in results likely emerges from the varied sample sizes and patient characteristics, the inclusion of patients with comorbid FTD, and diverging methodological approaches. In order to mitigate these problems, we explicitly included a clinically homogenous group without ALS-FTD patients, and used a whole-brain voxel-level

[3] The chapter is partially based on an article by Loewe, K.*, Machts, J.*, Kaufmann, J., Petri, S., Heinze, H.-J., Borgelt, C., Harris, J. A., Vielhaber, S., Schoenfeld, M. A. 2015. Widespread temporal lobe dysfunction in amyotrophic lateral sclerosis. *Under review.* *Authors contributed equally.

approach in the analysis. This analysis technique has advantages as compared to previously used seed based approaches, as such approaches are often biased by seed selection or mismatch of functional and region-of-interest (ROI) boundaries. We hypothesize that, at the level of individual voxel pairs, we will detect a pattern of neuronal degeneration within the motor system, as well as other functional systems that are usually involved in FTD with regard to the continuum hypothesis.

5.2. Methods

5.2.1. Participants

We studied 64 patients with ALS and 38 healthy controls. Patients were classified according to the revised El Escorial Criteria (Brooks et al., 2000) and clinical severity was rated using the revised ALS Functional Rating Scale (ALSFRS-R, Cedarbaum et al., 1999). To maximize clinical homogeneity, patients with progressive muscular atrophy, primary lateral sclerosis, or concomitant FTD were not included in this study. A diagnosis of FTD was made based on current diagnostic criteria (Gorno-Tempini et al., 2011; Rascovsky et al., 2011) and included the interview of the caregiver. Exclusion criteria for all participants included cerebrovascular disease, traumatic brain injury, and other neurologic or psychiatric diseases. Moreover, the controls were screened for cognitive impairment and were included only if they scored within the normal range of the Montreal Cognitive Assessment (Nasreddine et al., 2005). The local ethics committee of the Otto-von-Guericke University, Magdeburg, Germany, approved the study, and all participants gave written informed consent prior to inclusion. Demographic data are summarized in Table 8.

5.2.2. Neuropsychological assessment

All participants underwent detailed neuropsychological assessment to test executive, memory, language, and visuo-spatial function. Executive function was tested using the Trail Making Test (TMT, Reitan, 1992), the Regensburger Verbal Fluency Test (RWT, Aschenbrenner et al., 2000), a computerized version of the Stroop Test (Stroop, 1935), and the backward digit span task from the revised Wechsler Memory Scale (WMS-R, Härting et al., 2000). To assess memory function, the forward digit span task (Härting et al., 2000) and the German version of the Rey Auditory Verbal Learning Test (VLMT, Helmstaedter et al., 2001) were employed.

Semantic language function was assessed using the Bogenhausen Semantic Test (BOSU, Glindemann, 2002), and visuo-spatial skills were tested using the copy subtest of the Rey Complex Figure Test (RCFT, Osterrieth, 1944). A detailed description of the neuropsychological test battery can be found in section 2.2.2. Tests were adapted to speech and motor deficits by analyzing tempo-independent (TMT and Stroop ratio) or tempo-adapted (fluency indices) scores. Nevertheless, due to the range of physical disabilities in the ALS cohort, not all patients were able to complete all tests (Table 9). In order to compare test results across domains, standardized Z scores were calculated for each neuropsychological tests by measures of healthy control mean and standard deviation (Z = [participant's score – healthy control mean]/healthy control standard deviation). All measures were adjusted such that a negative Z score indicates worse performance.

The distributions of demographic and neuropsychological variables were tested for normality using the Kolmogorov-Smirnov test. Differences in education, gender, and handedness were assessed using chi-square (gender, handedness) and Mann-Whitney *U* (education) tests. Age was normally distributed and between-group differences were tested using an independent two-sample *t* test. Neuropsychological test variables were not normally distributed, and between-group differences were assessed using the non-parametric Mann-Whitney *U* test. To demonstrate the full range of impairment, Table 9 indicates the number of impaired patients and healthy controls in each individual test. Cut-off values are based on a Z score less than -2.

Tab. 8: Demographic profile of participants

Group	No.	Age	Sex (male-female)	Handedness (right-left)	Education (years)	limb-bulbar onset	ALSFRS-R	Disease duration (months)
HC	38	59.5±10.2	26-12	38-0	11.1±1.0	na	na	na
ALS	64	58.9±11.5	39-25	59-5	10.6±1.1	46-18	38.9±5.7	20.4±15.0
p Value		0.77	0.45	0.08	0.06	-	-	-

5.2.3. MRI data acquisition

All imaging data were acquired on a Siemens Magnetom VERIO 3T MRI scanner (Siemens, Erlangen/Germany) with a 32-channel head coil. A high-resolution, T1-weighted structural scan was obtained for anatomical reference using a 3D-MPRAGE sequence (TE = 4.82ms,

TR = 2500ms, TI = 1100ms, flip angle = 7°, voxel size = 1mm³). Resting-state fMRI scans were acquired using an echo-planar imaging (EPI) sequence (TE = 30ms, TR = 2200ms, flip angle = 80°, voxel size = 3.5mm^3) sensitive to blood oxygen level-dependent (BOLD) contrast. Slices were obtained parallel to the intercommissural (AC-PC) line. Participants were instructed to close their eyes and remain awake during acquisition. To address the problem of geometric distortions in EPI caused by magnetic field inhomogeneity, a B0 field map was acquired prior to the EPI sequence using a double-echo gradient recall echo (GRE) sequence (TE 1/2 = 4.92ms/7.38ms, TR = 675ms, flip angle = 60°, voxel size = 2.6×2.6×2.0mm³).

5.2.4. MRI data preprocessing

Kristian Loewe performed the MRI data preprocessing using the FMRIB Software Library (FSL). Structural images were skull-stripped (Smith, 2002) and warped to MNI space using FLIRT (Jenkinson et al., 2002) and FNIRT (Andersson et al., 2010). Functional images were slice time corrected and realigned to the mean functional image to compensate for head motion (Jenkinson et al., 2002). Geometric distortions induced by magnetic field inhomogeneity were corrected for based on the GRE field map (EPI distortion correction). Data were registered to the corresponding structural scan and warped to the MNI space. The resulting standard-space images were spatially smoothed using a Gaussian kernel (7mm FWHM) to improve the signal-to-noise ratio and to further accommodate inter-individual anatomic variations. Finally, to account for low frequency intensity drifts and high frequency noise, the data were bandpass-filtered (0.01-0.1Hz).

5.2.5. Functional connectivity analysis

Preprocessed data were used for graph analysis (for detailed review on graph analysis see (Bullmore and Sporns, 2009; Misic et al., 2015). A graph is a mathematical representation of interconnected elements and its structure is comprised of a set of nodes and edges (Sporns, 2011), where nodes represent the elements of a given network, and edges the connections between the pairs of nodes. Various systems can be modeled as a graph, such as flight patterns, social networks or neurons of the brain, where each neuron represents a node and the interconnections between these neurons represent edges within the graph. A graph is best represented in a connection matrix (adjacency matrix), where nodes are represented in matrix

rows and columns, and edges are represented as binary or weighted matrix entries (Sporns, 2011). Brain network connectivity can be described by either anatomical tracts or functional associations between these regions (Rubinov and Sporns, 2010). Here, we used resting state fMRI data to assess functional connectivity changes using a custom graph-based voxel-level approach developed in our group. Individual connectivity graphs were constructed by defining gray matter voxels as nodes and establishing weighted edges by estimating the internodal correlation between the nodes' associated time series using Pearson's *r*. Edge-level *t* statistics were computed across graphs to assess between-group differences in connectivity. The resulting graph of statistics G_t was used to derive the graph of *p* values G_p (Leventhal and Huynh, 1996). G_p was then pruned based on *q* value estimation (Storey and Tibshirani, 2003) in order to identify edges, i.e., pairs of voxels, that exhibit significant differences (FDR < 0.05). Based on their direction (Leventhal and Huynh, 1996), the so-obtained graph G_s formed by the remaining edges was partitioned into the two subgraphs $G_{s<}$ and $G_{s>}$ corresponding to ALS-related decreases and increases in connectivity, respectively.

To visualize $G_{s<}$ and $G_{s>}$, we used circos (Krzywinski et al., 2009) to generate connectograms (Irimia et al., 2012) that were specially designed to accommodate the illustration of voxel-level information. Specifically, each circular segment in a voxel-level connectogram corresponds to a region of the Harvard-Oxford atlas (Desikan et al., 2006) and each voxel is assigned a unique position on the segment of its comprising region using multidimensional scaling based on the pairwise similarity of the voxels' connectivity profiles with respect to G_t. A link connecting two voxels indicates significantly decreased or increased functional connectivity in ALS as compared to controls for these voxels.

5.3. Results

5.3.1. Neuropsychology

Neuropsychological deficits in ALS patients were limited to the executive and the verbal memory domains (Figure 14). More specifically, performance deficits were observed in letter flexibility (U = 706.0, p = 0.045), Stroop effect (U = 577.0, p = 0.004) and error rate (U = 687.5, p = 0.030), cognitive flexibility (U = 692.5, p = 0.022), and recognition memory (U = 767.0, p = 0.028). There were no between-group differences in letter fluency (U = 757.5, p = 0.067), semantic fluency (U = 789.0, p = 0.192), and semantic flexibility (U = 759.0, p = 0.119), visuo-spatial skills (U= 380.5, p = 0.139), semantic categorization (main features:

Tab. 9: Group-specific neuropsychological performance.

	ALS (Mean±SD)	N	HC (Mean±SD)	N	Cut-off value	Nr. of impaired controls	Nr. of impaired patients
Verbal Learning	48.78±10.7	55	50.71±8.4	38	33.91	1	4
Immediate recall	9.91±3.4	55	10.68±2.8	38	5.08	2	6
Delayed recall	9.85±3.4	55	10.55±3.0	38	4.55	3	4
Recognition	10.62±4.5	55	12.50±2.8	38	6.90	2	9
Digit span forward	7.35±1.8	60	7.45±1.6	38	4.25	1	2
Letter fluency	4.82±2.3	53	3.91±1.4	37	6.71	2	8
Letter flexibility	5.96±4.4	51	4.34±1.5	37	7.34	2	10
Semantic fluency	2.32±1.0	51	2.03±0.7	37	3.43	1	3
Semantic flexibility	3.87±1.7	51	3.35±0.9	37	5.15	2	5
Stroop effect	1.20±0.3	49	1.06±0.2	37	1.46	0	3
Stroop errors	2.1±4.2	49	0.54±1.0	37	2.54	1	11
Cognitive flexibility	2.72±1.2	51	2.26±0.8	38	3.86	2	6
Digit span backwards	5.70±1.6	60	6.32±1.6	38	3.12	1	3
Semantic categorization – main features	9.90±0.3	61	9.87±0.4	38	9.07	4	6
Semantic categorization – sub features	9.13±0.9	61	9.16±1.0	38	7.16	4	4
Visuo-spatial skills	31.47±3.5	36	32.80±2.5	27	27.80	1	4

$U = 1148.0$, $p = 0.880$; sub features: $U = 1124.0$, $p = 0.787$), digit span forward ($U = 1090.0$, $p = 0.710$) and backward ($U = 893.5$, $p = 0.065$), verbal learning ($U = 935.0$, $p = 0.390$), as well as immediate ($U = 918.5$, $p = 0.320$) and delayed ($U = 926.0$, $p = 0.349$) recall. Means, standard deviations, and the percentage of impaired patients and controls in each neuropsychological test are displayed in Table 9.

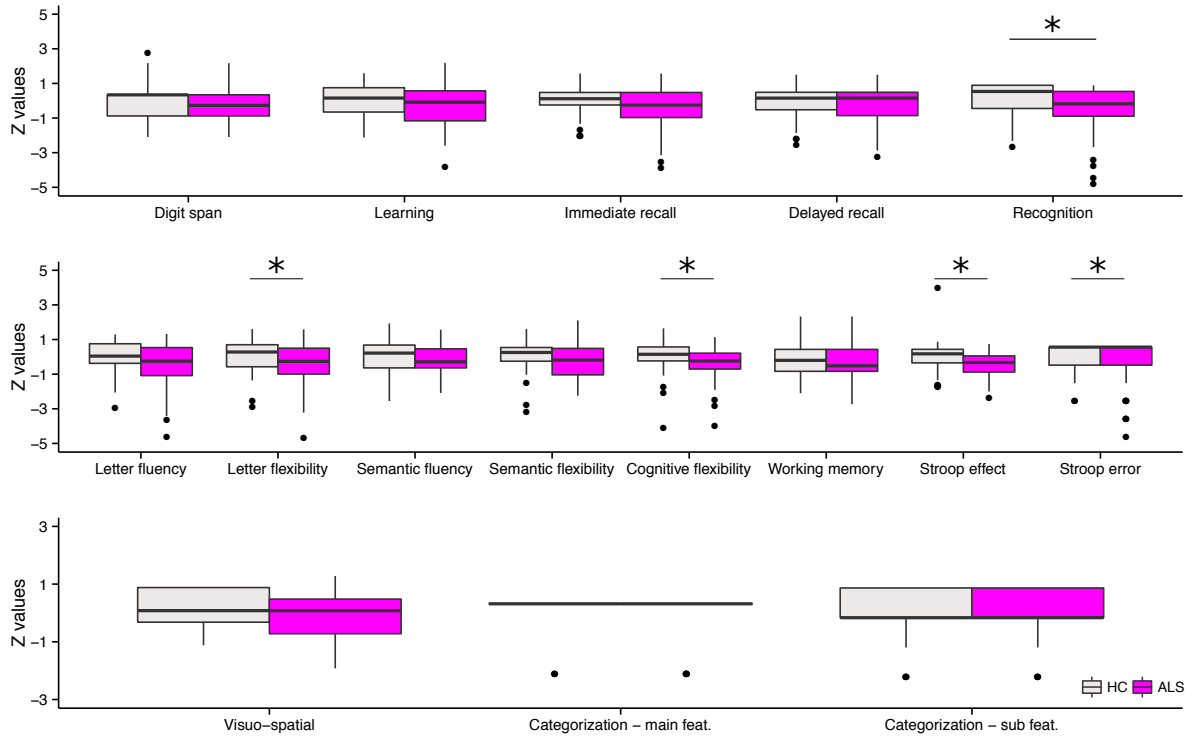

Fig. 14: Distribution of standardized neuropsychological performance in memory (top), executive function (middle), visuo-spatial and language (bottom) domains. The boxes represent the median, first, and third quartile; the whiskers represent +/- 1.5* the interquartile range. Z scores are standardized by healthy control mean and standard deviation. * $p < 0.05$

5.3.2. Functional connectivity

The whole-brain voxel-level graph analysis revealed complex patterns of both decreased and increased connectivity in ALS compared to controls (Figure 15). Specifically, clusters of reduced functional connectivity were observed in cortical sensorimotor (bilateral pre- and postcentral gyrus), parietal (left superior parietal, right angular gyrus), temporal (bilateral temporal pole, left planum temporale, bilateral superior and inferior temporal gyrus, right middle temporal gyrus, bilateral parahippocampal gyrus and fusiform cortex), occipital (bilateral fusiform cortex, lateral occipital cortex, cuneal cortex, lingual gyrus, occipital pole and intracalcarine cortex, right precuneus, right supracalcarine cortex), frontal (bilateral frontal pole, left insular cortex, right middle frontal gyrus, right operculum cortex), and subcortical regions (bilateral thalamic nuclei, left amygdala, bilateral hippocampi, bilateral caudate nuclei, left nucleus accumbens). Focusing on the motor system, patients with ALS showed impaired long-range functional connectivity between primary motor and sensorimotor regions (pre- and postcentral gyrus) and the occipital pole, affecting both intra- and interhemispheric connections (Figure 16). Along with the motor system another functional

system exhibited extensive changes in connectivity: the temporo-occipital cortex. Specifically, ALS-related connectivity decreases were observed between temporal and occipital lobe areas, incorporating short-range connections as well as interhemispheric connections. Functional connectivity was also decreased between cortico-subcortical areas, involving connections among bilateral hippocampi and occipital lobe areas as well as the right thalamus and left temporal lobe areas.

While less pronounced, the analysis also revealed clusters of increased connectivity associated with ALS (Figure 15), located in cortical sensorimotor (right pre- and postcentral gyrus), frontal (left inferior frontal gyrus, left insular cortex, bilateral frontal pole), temporal (left temporal pole, left planum polare, right fusiform gyrus), occipital (bilateral lateral occipital cortex, left precuneus), parietal (right angular gyrus), and subcortical regions (right hippocampus). Further connectivity increases were observed between right parietal lobe and left temporal pole as well as right occipital lobe and the frontal lobes. These clusters result mainly from increased interhemispheric connectivity (Figure 16).

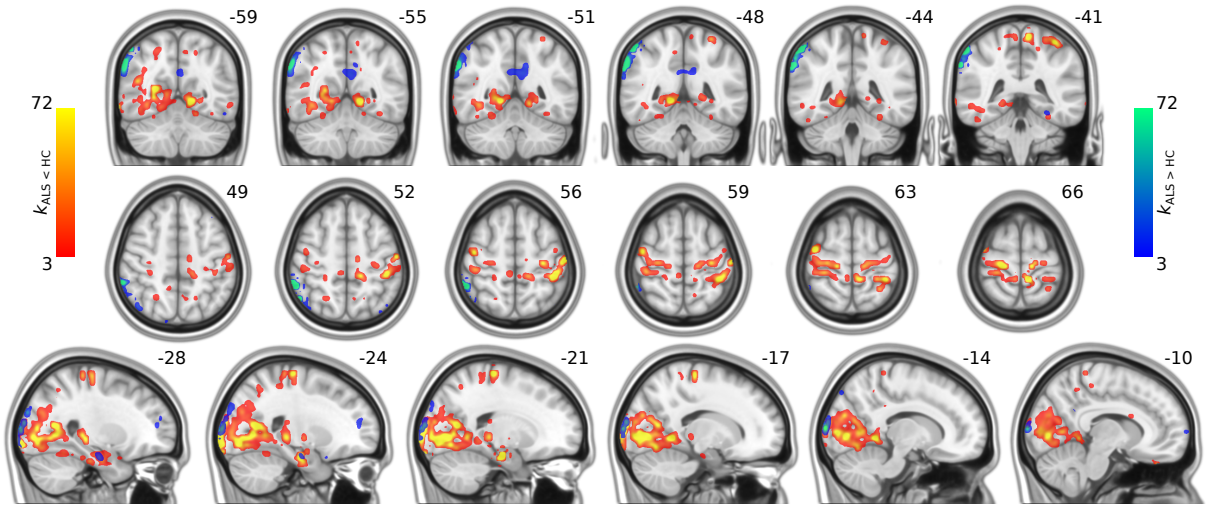

Fig. 15: Clusters of decreased and increased functional connectivity in ALS patients compared to healthy controls. Degree maps $k_{ALS < HC}$ and $k_{ALS > HC}$ are superimposed on top of MNI slices in order to map clusters of decreased and increased connectivity in ALS, respectively. The degree $k_{ALS < HC}$ ($k_{ALS > HC}$) of a voxel/node v is the number of pairs (v,w) in G_t, exhibiting significantly decreased (increased) functional connectivity, where w can be any other voxel than v (FDR < 0.05).

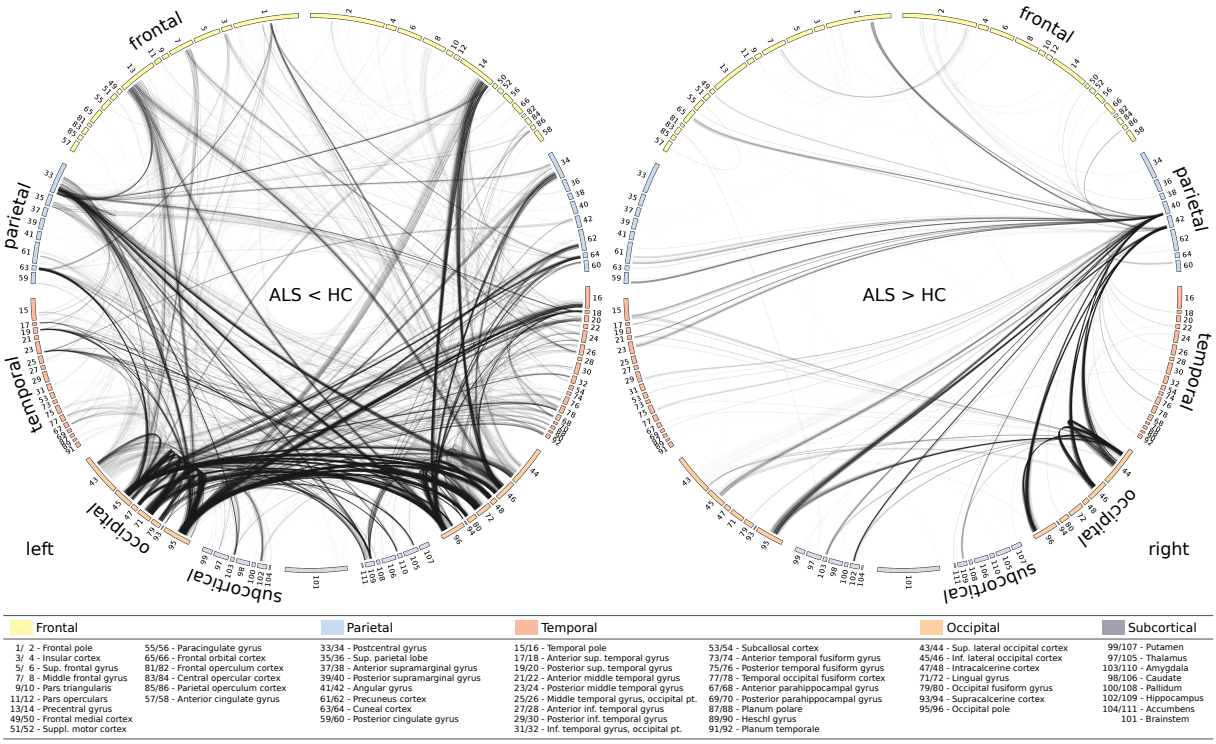

Fig. 16: Voxel-level connectograms. Each circular segment corresponds to a region from the Harvard-Oxford atlas. Each voxel was assigned a unique position on the segment of its comprising region using multidimensional scaling based on the pairwise similarity of the voxels' connectivity profiles with respect to G_t. A link connecting two voxel indicates significantly decreased or increased functional connectivity in ALS (FDR < 0.05).

5.4. Summary

The present study investigated the neural correlates of degeneration in ALS patients. Using functional MRI at rest in conjunction with a whole-brain voxel-level approach, we examined functional connectivity alterations in ALS patients. Consistent with the hallmark pathology of the disease, we observed prominent clusters of decreased functional connectivity in motor-related areas, which were predominantly characterized by many affected long-range connections. Strikingly, the analysis also revealed widespread patterns of decreased functional connectivity along the temporal and occipital lobes, a pattern normally observed in FTD patients. This is especially of importance since ALS-FTD patients were explicitly excluded from the study, and the remaining ALS patients exhibited only minor cognitive deficits, if any at all. The current results provide in-vivo evidence for the involvement of the temporal lobe in non-demented ALS patients, supporting recent genetic (DeJesus-Hernandez et al., 2011; Renton et al., 2011), and histopathological (Neumann et al., 2006) reports pointing to a shared pathology between ALS and FTD.

6. General discussion

In the following chapter, the results from Studies 1-4 will be discussed in separate sections, with a general discussion and a corresponding summary presented at the end.

6.1. Memory impairment in ALS differs from that observed in Alzheimer's Disease

This cross-sectional study of neuropsychological performance in controls, ALS, and amnestic mild cognitive impairment (aMCI) patients revealed a significant deficit in verbal memory function in both patient groups relative to controls. In patients with ALS we observed additional dominant executive deficits including the impairment of verbal fluency, which is a consistent, well-documented finding in motor neuron disease (Abrahams et al., 2005; Abrahams et al., 2000; Phukan et al., 2012; Phukan et al., 2007). This deficit also has an impact on disease progression (Elamin et al., 2013) and survival (Elamin et al., 2011). In contrast, previous reports on memory dysfunction are more inconsistent. When evaluating overall memory impairment as a composite parameter of several memory subdomains, there were no differences between aMCI and ALS. However, examining the nature of amnestic deficits based on more predefined sub-function revealed disease-group specific patterns of impairment.

Patients with aMCI had overt deficits in short and delayed recall, whereas ALS patients were mainly impaired in verbal memory recognition. Recall deficits in aMCI are a hallmark feature of this patient group, as it is known to be a pre-Alzheimer´s Disease stage (Winblad et al., 2004). In contrast, recall performance in patients with ALS was not affected. Previous studies on ALS that took verbal memory into account have reported both impaired and preserved recall performance on word-list learning tests, depicting the inconsistency among memory deficits in ALS. One study reported long-delay recall impairment but no impairment on short-term recall or recognition (Hanagasi et al., 2002), while others found deficits in short-term recall with preserved delayed recall performance (Massman et al., 1996), or impairment in both short and delayed recall (Christidi et al., 2012). Here, we show that memory deficits in ALS seem to be disease specific and can be differentiated from other neurodegenerative diseases such as AD when assessed with precise measures.

Recognition deficits in patients with ALS were found to be the most evident memory deficit and have been reported before (Munte et al., 1998; Raaphorst et al., 2011). In experimental psychology, recognition memory is understood as a 'dual-process' model that incorporates the product of two different memory functions, namely familiarity and recollection (Rugg et al., 1994). Thus, recognition judgments can be based on the recollection of details about previous events or on the assessment of stimulus familiarity (Yonelinas, 2002). In ALS, only one study so far investigated recognition memory and its underlying neuronal mechanisms (Munte et al., 1998). The paradigm consisted of a verbal recognition task, where the subjects had to decide whether a presented word had been presented previously. Although this was not a standardized test, results can be compared to the recognition test that was used in this study (VLMT). Similar to our observations, Muente et al. (1998) reported a recognition deficit in patients with ALS that manifest as the absence of a recognition associated event-related potential. Interestingly, this effect was not observed in patients with Alzheimer's Disease who completed a comparable task (Rugg et al., 1994). These results support the different qualities of memory impairment in ALS and AD observed in the current study.

Although ALS-related recognition deficits have been mostly associated with executive dysfunction (Christidi et al., 2013; Raaphorst et al., 2011), our results show that executive impairment could only account for 20.5% of memory performance in ALS. This finding supports the emerging notion that executive function is only one of several cognitive domains impaired in ALS (Abrahams, 2013; Phukan et al., 2012). Moreover, executive dysfunction in frontotemporal dementia has been related to failure in source memory but not recognition performance (Simons et al., 2002), which leads to the assumption that the recognition deficit reported here cannot be caused solely by executive dysfunction. Impairment in recognition can emerge from either insufficient encoding as a temporal lobe function or from deficient prefrontal cortical function. However, both assumptions would lead to a higher amount of errors and interference biases, which was more pronounced in patients with ALS than in patients with aMCI.

In addition to detailed neuropsychological testing and/or neurophysiological measures, imaging studies can provide more information about the specific anatomical structures involved. A number of studies correlated cognitive performance with white or grey matter integrity (Christidi et al., 2013; Lillo et al., 2012; Sarro et al., 2011; Schuster et al., 2014), but only two focused on word list learning and structural integrity. The results, however, seem

inconsistent since one reported a relationship between memory performance in ALS and uncinate fasciculus (UF) integrity (Christidi et al., 2013), whereas the other study found no such association (Sarro et al., 2011). Since both studies had relatively small sample sizes, further structural-functional correlations are needed to draw more definite conclusions on the relationship between memory impairment and cerebral pathology. Given that the uncinate fasciculus connects temporal lobe structures such as the hippocampus with frontal lobe areas, its involvement highlights the contribution of structures other than the frontal lobe in memory performance in ALS. Hippocampal and parahippocampal pathology in ALS are well described in post mortem studies (Brettschneider et al., 2013; Takeda et al., 2009; Takeda et al., 2007), and lesions have been related to amnestic deficits. Interestingly, those lesions were different from those found in Alzheimer´s Disease (Takeda et al., 2007), which underscores the distinct neuropsychological profiles between patients with ALS and aMCI in the present study.

This study measured a wide range of cognitive domains, including verbal memory, executive functions, and visuo-spatial skills, with standardized neuropsychological tests. Tests of verbal memory revealed substantial recognition deficits in patients with ALS. However, there are some limitations that should be taken into account. Initially, we did not have a specific hypothesis as to which sub-function of memory would be different in ALS and aMCI, and we therefore chose a memory test that covers several sub-functions (VLMT). With the results presented here, further research should focus on recognition memory, both verbal and visual, and investigate if the observed deficits are caused by deficient familiarity or recollection performance. It would also be of interest to relate it to prefrontal lobe dysfunction, either measured by behavioral or imaging parameters, in order to identify correlates of impaired recognition in ALS.

In conclusion, the current investigation suggests that memory impairment in ALS is different from that observed in preclinical Alzheimer´s disease, but also that there is considerable overlap in verbal memory performance between these conditions. The differentiation between the distinct cognitive profiles of the two patient groups can only be captured with detailed subdomain specific neuropsychological testing, and composite scores of domain level performance proved insufficient. Moreover, this study further underscores the considerable extra-motor deficits in ALS that extend well beyond executive dysfunction. Emerging

cognitive screening tests in ALS, which are increasingly used in clinical trials and specialist clinics should take the unique memory deficits of ALS in to consideration.

6.2. Hippocampal shape analysis reveals local shape deformations in the CA1 region

Study 2 was designed to identify hippocampal volume and shape characteristics in a representative group of patients with ALS compared to healthy controls. Using both a manual and automatic segmentation approach, we identified global hippocampal volume loss associated with ALS that was complemented by local shape deformations in the CA1 region located in the hippocampal head.

The volumetric analysis revealed gray matter volume loss associated with ALS in the right and left hippocampus (Figure 4). The reported volume reductions were irrespective of using manual or automatic segmentation tools. Although mean differences in left hippocampal volumes between ALS patients and healthy controls were comparable to those of the right hippocampus, variances of left hippocampal volumes were slightly higher and the significance level of $p < 0.05$ was not reached. However, the smaller effect in the left hippocampus does not point to a meaningful lateralization effect. It is rather the assumption of a global hippocampal volume reduction that is further supported by previous neuroimaging studies, where, depending on the patient cohort, either the left (Bede et al., 2013c), right (Abdulla et al., 2014), or bilateral (Westeneng et al., 2015) hippocampal volume was significantly reduced in ALS. Taken together, the reported volume reductions from this study are in line with previous reports (Abdulla et al., 2014; Bede et al., 2013c; Westeneng et al., 2015) and promote the concept that the disease substantially affects the hippocampus.

In addition to hippocampal volume loss, we identified ALS-related shape deformations in the hippocampal head using a vertex-wise approach. Unlike volumetric measures, the conducted shape analysis do account for the heterogeneity of the hippocampal formation, i.e., the cytoarchitectonic subfields and the anterior-posterior functional segregation along the longitudinal axis (Poppenk et al., 2013). Here, shape deformities were found in a region corresponding to the cornu ammonis field 1 (CA1), where pyramidal cells project either directly or via the subiculum to the cortex (Van Hoesen and Hyman, 1990). This region is

also known to be the primary output region of the hippocampus, and it receives its main input from CA3 neurons through Shaffer collaterals and the entorhinal cortex (Small et al., 2011). On a functional level, CA1 is found to be critically involved in successful encoding and retrieval of long-term memory (Duncan et al., 2014), as well as in novelty detection (Lisman and Grace, 2005). Recently, a functional MRI study investigated novelty-related hippocampal activity in a group of ALS patients over the course of three months (Stoppel et al., 2014). Compared to healthy controls, ALS patients showed no alterations of hippocampal activation during the presentation of novel stimuli. Interestingly, when repeating the experiment after 3 months, patients with ALS showed a significant increase in hippocampal activity while the behavioral performance was identical to the initial measurement (Stoppel et al., 2014). The authors interpreted this effect as a mechanism to compensate for the beginning of structural lesions, which has been observed previously in other neurodegenerative conditions (Bookheimer et al., 2000). The results of the present study support this hypothesis and provide a structural correlate for the reported functional alterations in the CA1 region in ALS.

In addition to the cytoarchitectonic segregation, another functional specialization within the hippocampal formation has been postulated (Moser and Moser, 1998; Poppenk et al., 2013). Input information to the entorhinal cortex is organized in an anterior-posterior gradient (Maass et al., 2015), which is preserved throughout other hippocampal subfields (Small et al., 2011). Within this framework, anterior parts of the hippocampal receive input from the amygdala and limbic system, while the posterior parts receive input from the visual cortex. Findings from functional MRI studies further support this organization and suggest that the anterior parts of the longitudinal axis are more engaged in emotional regulation whereas posterior regions are more involved in memory and spatial cognition (Fanselow and Dong, 2010; Robinson et al., 2015). Our findings indicate that ALS-related hippocampal pathology is primarily located in the anterior parts of the structure, thus affecting mainly the limbic system and associated functions. Recent population-based studies have shown that behavioral deficits such as apathy, stereotypies, and disinhibition are a prominent feature of ALS (Gibbons et al., 2008; Grossman et al., 2007; Lillo et al., 2011; Lillo et al., 2012), can even precede motor symptoms (Mioshi et al., 2014a), and are likely to be a consequence of the identified CA1-lesion. Apart from behavioral deficits, Study 1 demonstrated that memory impairment in ALS can be a feature of cognitive dysfunction, although it is different from a pure amnestic deficit frequently observed in Alzheimer's Disease. The findings of the current study present a structural correlate of the observed differences in memory profiles in Study 1

and lead to further questions regarding the role of the hippocampal formation and its connections in ALS pathology.

One of the limitations of the present study is the applied magnetic field strength of 1.5T. Although the resolution of the T1 images was high, it is difficult to precisely delineate the border between two contiguous subfields, even at higher resolution/field strength (de Flores et al., 2015), and conclusions should therefore be drawn cautiously. Nevertheless, results from histological studies confirm the differential vulnerability of CA1 neurons to excitotoxicity, which has been reported to play a critical role in ALS pathogenesis (see chapter 1.3).

Taken together, the current results provide evidence for hippocampal involvement in ALS, which is characterized by global volume loss and local atrophy in the CA1-region and represent a neuronal correlate for the cognitive and behavioral deficits frequently encountered in the disease.

6.3. Subcortical pathology as a function of neuropsychological deficits

The results of Study 3 provide evidence of increasing subcortical gray matter pathology along the cognitive phenotypes of ALS, ALS-Plus, and ALS-FTD, suggesting that the functional continuum proposed by neuropsychological studies is driven by an underlying pathological continuum. Moreover, vertex analyses in ALS-FTD revealed preferential atrophy of sub-regions within the basal ganglia, which connect to established cortical sites of ALS pathology and suggest a network-wise vulnerability of interconnected gray matter regions.

Our results indicate that hippocampal and thalamic volume reductions are the most sensitive predictors of cognitive phenotype classification. As pointed out in section 6.2, hippocampal pathology is a well-documented feature of ALS (Takeda et al., 2009) and corresponds with stage 4 of the recently proposed pTDP-43 pathological staging system (Brettschneider et al., 2013). Hippocampal atrophy has been previously linked to memory deficits in ALS (Abdulla et al., 2014), and hippocampal dysfunction seems to manifest later than motor dysfunction (Stoppel et al., 2014). Here, we confirm both volumetric and vertex-wise hippocampal changes, and demonstrate that hippocampal atrophy is closely associated with verbal memory performance. While ALS patients without cognitive impairment showed no significant

hippocampal changes, ALS-Plus patients exhibited volume loss in the head of the hippocampi. A similar anatomical pattern was observed in ALS-FTD, but with a higher degree of volume loss, and surface-projected changes expand from the head of the hippocampus to the body of the structure. Our findings underscore the importance of cognitive assessment beyond executive function in ALS.

Thalamic atrophy was identified as another sensitive discriminator of cognitive categorization, and vertex analyses revealed a distinct pattern of thalamic involvement in ALS-FTD. These changes preferentially affect the anterior nuclei, which have projections to the limbic system, especially to the hippocampus and cingulate gyrus. This anatomical vulnerability corresponds with the prominent behavioral deficits observed in ALS-FTD. Additional atrophy was observed in the anterior ventral and lateral thalamic nuclei, which are known to project to motor and supplementary motor regions. Motor cortex pathology is a hallmark feature of ALS (Bede et al., 2013a); therefore the degeneration of associated thalamic nuclei is not surprising. These observations warrant further studies of thalamocortical connectivity to explore its contribution to motor planning and disability in ALS.

Caudate nucleus pathology in ALS-FTD affected both the head and tail of the structure. The head of the caudate receives input from the dorsolateral prefrontal cortex (Alexander et al., 1986), a region directly linked to executive function (Poston and Eidelberg, 2012). The tail of the caudate is associated with visuo-spatial information processing (Lawrence et al., 2000), which is known to be impaired in ALS (Phukan et al., 2012) and deteriorates with disease progression (Elamin et al., 2013). Moreover, the caudate nucleus is strongly interconnected with the putamen, where, in turn, dorsal atrophy was also observed. Dorsal putaminal regions receive afferentation from the motor-, premotor-, and supplementary motor cortices, all of which are heavily affected in ALS (Bede et al., 2013a). Interestingly, other studies of subcortical pathology in ALS did not capture changes in the putamen (Bede et al., 2013c; Westeneng et al., 2014), which is likely to be explained by their unsegregated patient cohorts. In addition to dorsolateral- and motor-related frontostriatal pathology, considerable changes were also identified in the accumbens nuclei, a structure that primarily receives input from the anterior cingulate cortex (Alexander et al., 1986). Accumbens pathology in ALS has been previously linked to the *C9orf72* genotype (Bede et al., 2013c) and used as a stage-defining feature of the pTDP-43 histopathology staging system (Brettschneider et al., 2013). The

nucleus accumbens is widely interconnected with the ventromedial prefrontal cortex (O'Callaghan et al., 2014), mediates motivation and plays a key role in reward-seeking behavior (Elamin et al., 2013). Consistent with its role in motivation, an association has been identified between accumbens atrophy and apathy measures.

Taken together, a distinctive pattern of pathology was observed within basal ganglia sub-regions, which are directly connected to the prefrontal, motor, and orbitofrontal cortex, i.e., key cortical regions of ALS pathology. The identified anatomical vulnerability of interconnected cortical and subcortical gray matter regions further supports the concept of network degeneration in ALS. Importantly, the observed imaging changes were independent from both disease duration and motor disability (ALSFRS-R) and are not merely a function of prolonged disease duration or higher disease burden.

Density analyses revealed cerebellar gray matter changes in *C9orf72*-negative ALS-Nci (Figure 10a). To date, cerebellar changes have been mostly associated with the *C9orf72* hexanucleotide repeat expansion both in ALS and FTD (Mackenzie et al., 2014). However, recent imaging studies have reported white matter pathology in non-demented *C9orf72*-negative ALS patients (Bede et al., 2015) and cerebellar gray matter atrophy along the ALS-FTD spectrum (Tan et al., 2014). Our whole-brain analyses highlight widespread cortical and basal ganglia pathology in ALS-FTD, not only in comparison to controls but also to ALS-Nci and ALS-Plus patients. While the extent of cortical gray matter atrophy in ALS-FTD (Figure 10b/c) is consistent with previous reports (Mioshi et al., 2013), basal ganglia involvement has not been previously characterized in this phenotype. We have identified considerable gray matter density reductions in the thalamus, caudate, putamen, and pallidum in ALS-FTD complementing our volumetric and vertex findings. These results are consistent with an imaging study of FTD without ALS (Garibotto et al., 2011).

One of the limitations of our study is the relatively small numbers of ALS-FTD and ALS-Plus patients. However, the observed anatomical changes were consistent across multiple imaging methods and demonstrated in volumetric, shape and density analyses. There is relatively little known about *C9orf72*-negative ALS-FTD, as landmark imaging studies of ALS-FTD (Chang et al., 2005) predate the discovery of *C9orf72* and are likely to have used admixed cohorts of hexanucleotide carriers and non-carriers. Studies published after the identification of *C9orf72* repeats on the other hand focus mainly on the imaging signature of *C9orf72*. Our study

characterizes a relatively small, but unique cohort of *C9orf72*-negative ALS-FTD patients. Another study limitation may stem from classifying patients into ALS-ci and ALS-bi based on the Strong criteria (Strong et al., 2009), which puts the emphasis on executive function, apathy and disinhibition for patient categorization. Recent reports, however, indicate that other cognitive domains, such as language (Abrahams, 2013), memory (Machts et al., 2014), and social cognition (Girardi et al., 2011) may also be pertinent in accurate patient categorization. Our finding of hippocampus mediated memory performance supports these observations and needs to be considered when revising the current consensus criteria.

The current findings indicate that the cognitive phenotypes of ALS are associated with incremental subcortical gray matter pathology that can be captured by volumetric, shape, and density measures in-vivo. The diagnoses of ALS-Nci, ALS-Plus, and ALS-FTD represent hierarchical categories along the same pathological continuum. The presented findings also provide evidence of a network-wise vulnerability of interconnected cortical and subcortical gray matter regions. Our results highlight that imaging studies of cognition in ALS and FTD need to assess basal ganglia integrity and not rely on cortical gray and subcortical white matter measures alone. The detailed characterization of basal ganglia pathology is pivotal to elucidate the frontostriatal changes underlying cognitive dysfunction in ALS.

6.4. Functional connectivity changes indicate widespread temporal lobe involvement

While Studies 2 and 3 focused on structural alterations in ALS in the temporal lobe, the last study investigated ALS-related functional connectivity changes. Consistent with the hallmark pathology of the disease, we observed prominent clusters of decreased functional connectivity in motor-related areas (Figure 15), which were predominantly characterized by many affected long-range connections (Figure 16). Strikingly, the analysis also revealed widespread patterns of decreased functional connectivity along the temporal and occipital lobes (Figure 15), a pattern resembling the neurodegenerative changes in FTD patients. This is of the utmost importance since ALS-FTD patients were explicitly excluded from the study, and our ALS patients exhibited only minor cognitive deficits, if any at all. The current results provide in-vivo evidence for the involvement of the temporal lobe in non-demented ALS patients, supporting recent genetic (DeJesus-Hernandez et al., 2011; Renton et al., 2011), and

histopathological (Neumann et al., 2006) reports pointing to a shared pathology between ALS and FTD.

The current results add to the growing body of literature providing a comprehensive account of the neural connectivity changes accompanying ALS pathology. A previous study from our group employed structural and functional MRI in conjunction with a longitudinal design and reported a drop in functional activity in the motor cortex reflecting the breakdown of compensatory mechanisms within only three months after diagnosis (Stoppel et al., 2014). This previous study also reported increased hippocampal activity across the same time range, presumably reflecting a mechanism of compensation (Stoppel et al., 2014). A second study employed DTI and found decreased structural connectivity in the motor system but increased diffusivity along occipito-temporal pathways (Steinbach et al., 2015). In the present study, we report prominent clusters of reduced functional connectivity in the motor cortex, completing the picture of pathological changes over the course of the disease. It would appear then, that ALS starts with subclinical neurodegenerative changes in the motor cortex, for which, at least in the very early stages, higher activity in non-affected motor neurons compensates (Agosta et al., 2011). Shortly after the first clinical symptoms appear, the neurodegeneration can no longer be compensated for, and a decay of upper and lower motor neuron function follows (Kimura et al., 2006), resulting in decreased BOLD activity in the motor cortex (Stoppel et al., 2014), as well as diffusivity along the pyramidal pathways (Steinbach et al., 2015).

The observed connectivity reductions in the motor cortex of ALS patients were primarily driven by affected long-range connections (occipital and temporal) to and from the motor cortex, and are in line with previously shown structural and functional changes in the motor system (Fekete et al., 2013; Verstraete et al., 2010; Verstraete et al., 2011). Functionally, the connectivity between motor areas and the visual cortex plays a key role in integrating visuo-motor information through cortico-cortical links (Glickstein, 2000; Goodale, 2011), and its observed degradation is likely a consequence of neuronal cell loss in primary motor regions. In sum, there is converging evidence from clinical and neurophysiological measures on the neurodegeneration and its temporal dynamics within the motor system. In terms of the temporal dynamics of disease progression, structural connectivity appear sensitive to alterations occurring over a longer time, whereas BOLD-related measures such as fMRI or measures of functional connectivity provide snapshots of ongoing dynamic processes evolving over shorter time periods. Therefore, depending on the time point of measurement,

they can show either increased (in the early subclinical stages) or decreased (in the more advanced clinical stages) values.

Since the typically studied ALS patient population is heterogeneous, and includes patients with comorbid frontotemporal dementia, the involvement of the temporal lobe as an integral component of the disease is less clear. As suggested by recent work (Agosta et al., 2011; Douaud et al., 2011), we excluded patients with ALS-FTD from the current study in order to address this issue. Importantly, we observed striking patterns of decreased functional connectivity across the occipital and temporal lobes. The extent of these patterns was surprising, especially given that the included patients exhibited only minor cognitive deficits, as well as the fact that all ALS-FTD patients were excluded. The connectivity decreases in the temporal lobe closely resemble the pattern of previously described functional network degeneration in patients with FTD (Agosta et al., 2013b) and speak in favor of a clear involvement of the temporal and occipital lobes in ALS, even in cognitively unimpaired patients. In light of common clinical (Phukan et al., 2012), genetic (DeJesus-Hernandez et al., 2011; Renton et al., 2011), and histopathological (Neumann et al., 2006) characteristics shared by ALS and FTD, these observations promote the concept of a single continuum upon which both conditions lie.

Importantly, similar patterns of connectivity reductions have also been observed in pre-symptomatic familial FTD (Dopper et al., 2013), suggesting that distinctive functional connectivity alterations might emerge before the first cognitive symptoms appear. A long pre-symptomatic period typically precedes the clinical phase of neurodegenerative disorders such as Parkinson's Disease (Stoessl, 2012) or Alzheimer's Disease (Langbaum et al., 2013), and has recently been proposed also for ALS (Eisen et al., 2014). In contrast to MRI-measures of structural connectivity (Steinbach et al., 2015), functional measures relying on the BOLD effect in the same data set were sensitive enough to detect small changes (Stoppel et al., 2014) and therefore to delineate the dynamics of pathology-related transient changes of neural activity in ALS. Here, our whole-brain connectivity approach detected ALS-related changes in the temporal lobe in a presumably pre-symptomatic phase, strongly suggesting that these changes reflect disturbances in neuronal homeostasis and functioning before cell-loss. In this context, connectivity-based biomarkers could facilitate earlier, perhaps even pre-symptomatic diagnosis and treatment.

In summary, the results presented here – widespread functional connectivity reductions in the temporal and occipital lobes in non-demented ALS patients – constitute compelling in vivo evidence for a shared pathology with FTD and pre-symptomatic connectivity changes in the temporal lobe. The analysis also revealed distinct, albeit relatively sparse, patterns of increased functional connectivity in the frontal and parietal lobes. These areas were recently reported to be part of an expanding disruption of structural connectivity spreading from primary motor areas to frontal and parietal regions over the course of 5.5 months (Verstraete et al., 2013). The observed functional connectivity increases could reflect regulatory processes that might serve to overcome the beginning structural lesion in the frontal and parietal lobes. These areas are also known to be involved in deficits in executive function and visuo-spatial processing frequently encountered in ALS (Agosta et al., 2013a; Phukan et al., 2007). In fact, the relatively minor neuropsychological executive deficits exhibited by the patients in the present study could indeed be a consequence of successful functional compensation. In support of this notion, we observed increased connectivity within the frontal, parietal, temporal, and occipital lobes (Figure 15). However, we also found increased interhemispheric connectivity, particularly between the parietal, temporal, and occipital lobes, possibly reflecting a loss of inhibitory GABAergic cortical interneurons (Maekawa et al., 2004; Turner et al., 2005) as a result of corpus callosum damage consistently reported in ALS (Douaud et al., 2011; Filippini et al., 2010). In summary, the observed patterns of increased connectivity seem better explained by a combination of both compensation and transcallosal disinhibition rather than by one of these mechanisms alone.

In combination, these findings – decreased structural (Verstraete et al., 2011) and functional connectivity in primary motor areas, increased functional connectivity in the frontal and parietal lobes, and decreased functional connectivity in the temporo-occipital lobe – suggest distinct ALS stages for the involvement of different functional networks. Extra-motor areas in the frontal and parietal lobes are affected at later stages of the disease than motor areas, and increases in functional connectivity can be interpreted as a mixture of compensatory mechanisms counteracting the evolving structural lesion (Agosta et al., 2013a) and transcallosal disinhibition (Douaud et al., 2011). Finally, the temporo-occipital cortex is targeted relatively late in the neurodegenerative process (Brettschneider et al., 2013; Kassubek et al., 2014), and though it is not yet affected by extensive cell loss, it already exhibits marked reductions in functional connectivity. It seems conceivable that these connectivity reductions emerge before the damage caused by progressive neurodegeneration

becomes observable through structural imaging methods. The current results provide novel insights into the pathophysiology of ALS and clearly indicate widespread temporal lobe involvement independent from FTD and support the idea of shared neurodegenerative pathological changes between ALS and FTD.

6.5. Summary

Amyotrophic Lateral Sclerosis shares clinical, genetic, and histopathological characteristics with frontotemporal dementia, leading to the hypothesis that both diseases represent opposite ends of one clinical continuum. Compared to the vast literature that reports on temporal lobe dysfunction in FTD as a hallmark of the disease, evidence regarding the involvement of the temporal lobe in ALS has been relatively sparse. Study 1 demonstrated that temporal lobe function is impaired in ALS, and this recognition memory impairment differs from a pure amnestic deficit of immediate and delayed recall performance usually observed in patients suffering from amnestic mild cognitive impairment. Importantly, these effects were not exclusively modulated by executive dysfunction, supporting an ALS-related temporal lobe dysfunction. By investigating the underlying anatomical structures of memory dysfunction, namely the hippocampal formation, Study 2 showed that atrophic changes within the hippocampus primarily target the CA1-region located at the hippocampal head. This region is primarily associated with novelty processing but also with deficient emotional regulation. Taken together, the results from Studies 1 and 2 provide evidence for a hippocampal involvement in ALS that is linked to the patients' neuropsychological and behavioral profile. These findings are further supported by Study 3, where the use of complementary imaging measures of volume, shape, and density showed a gradient of subcortical pathology that is closely associated with neuropsychological changes. Notably, the hippocampal volume differentiated best between different cognitive phenotypes of ALS, adding to the results of Studies 1 and 2. Finally, by investigating ALS-related functional connectivity changes, Study 4 revealed widespread patterns of decreased connectivity within the temporo-occipital lobe in non-demented ALS patients, that resembles the pattern of neuronal degeneration typically observed in FTD.

In summary, with the evidence that the temporal lobe can also be affected in ALS, the present data bolster the theory that there is a single disease continuum between ALS and FTD. This

was evident by means of neuropsychology, as well as structural and functional in vivo neuroimaging measures, and it highlights the importance of the characterization of the structural and functional organization of the temporal lobe, and its pathological involvement, in ALS.

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8. Publications

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Erklärung

Hiermit erkläre ich, Judith Wesenberg, geb. Machts am 20.06.1987 in Berlin, dass ich die von mir eingereichte Dissertation zum Thema:

“Temporal lobe pathology in Amyotrophic Lateral Sclerosis”

selbständig verfasst habe und die benutzten Hilfsmittel und Quellen vollständig angegeben wurden. Weiterhin erkläre ich, dass ich weder diese, noch eine andere Arbeit zur Erlangung des akademischen Grades doctor rerum naturalium (Dr. rer. nat.) an anderen Einrichtungen eingereicht habe.

Magdeburg, den 29.08.2016

Judith Wesenberg