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Hippocampal subfield and anterior-posterior segment volumes in patients with sporadic amyotrophic lateral sclerosis

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ABSTRACT

Neuroimaging studies of hippocampal volumes in patients with amyotrophic lateral sclerosis (ALS) have reported inconsistent results. Our aims were to demonstrate that such discrepancies are largely due to atrophy of different regions of the hippocampus that emerge in different disease stages of ALS and to explore the existence of co-pathology in ALS patients. We used the well-validated King's clinical staging system for ALS to classify patients into different disease stages. We investigated *in vivo* hippocampal atrophy patterns across subfields and anterior-posterior segments in different King's stages using structural MRI in 76 ALS patients and 94 health controls (HCs). The thalamus, corticostriatal tract and perforant path were used as structural controls to compare the sequence of alterations between these structures and the hippocampal subfields. Compared with HCs, ALS patients at King's stage 1 had lower volumes in the bilateral posterior subiculum and presubiculum; ALS patients at King's stage 2 exhibited lower volumes in the bilateral posterior subiculum, left anterior presubiculum and left global hippocampus; ALS patients at King's stage 3 showed significantly lower volumes in the bilateral posterior subiculum, dentate gyrus and global hippocampus. Thalamic atrophy emerged at King's stage 3. White matter tracts remained normal in a subset of ALS patients. Our study demonstrated that the pattern of hippocampal atrophy in ALS patients varies greatly across King's stages. Future studies in ALS patients that focus on the hippocampus may help to further clarify possible co-pathologies in ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with both clinical and hereditary heterogeneity (Swinnen and Robberecht, 2014). The aetiology of ALS is currently not known; however, interactions between genetic and environmental factors likely underpin disease susceptibility (Taylor et al., 2016). The onset of ALS involves a multistep process and has a long pre-symptomatic period (Chiò et al., 2018). In most patients with sporadic ALS, the main protein identified in cytoplasmic inclusions is phosphorylated 43 kDa transactive response

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DNA-binding protein (pTDP-43), which is also detected in nearly half of all patients with frontotemporal lobar degeneration and Alzheimer's disease (AD) (Josephs et al., 2014; Taylor et al., 2016). Similarly to AD patients, ALS patients may present with co-pathologies (Josephs et al., 2014; Hamilton et al., 2004; Jucke et al., 2018).

ALS is considered a multisystemic disorder in which almost half of patients present with varying degrees of cognitive deficits (Crockford et al., 2018; Eisen et al., 2017). PTDP-43 pathology in ALS can be divided into four stages (Braak stages): it begins focally, and then spreads persistently in sequential and regional patterns that typically originate from the motor cortex and extend to the prefrontal cortex, thalamus and eventually, the hippocampus (Braak et al., 2013). Recently, diffusion tensor imaging (DTI)-based fibre tracking studies have been used to further verify these pathological stages *in vivo* (Kassubek et al., 2014).

The hippocampus is not a uniform structure and comprises multiple cytoarchitectonically-defined subfields (Hainmueller et al., 2020). Neuronal populations located at distinct portions along the anterior-posterior axis of the hippocampus are associated with specific functions and are incorporated into different cortical networks (Ritchey et al., 2020). Furthermore, under neurodegenerative conditions, different pathological inclusions may preferentially affect specific hippocampal subfields and/or segments along the longitudinal axis (de Floreset al., 2020; Lladó et al., 2018). Indeed, various abnormal protein aggregates have been shown to correlate with distinct spatial patterns of hippocampal atrophy (Yokota et al., 2020). TDP-43 pathology may exert preferential vulnerability of the dentate gyrus (DG), and tau pathology may also have specific influence on the subiculum along the AD continuum (Lladó et al., 2018; Yokota et al., 2020). Specifically, similar spatial patterns have been identified in the hippocampus of ALS patients, and evidence has consistently demonstrated that the earliest and most frequently affected hippocampal subfield associated with TDP-43 pathology in ALS patients is the DG, which can be further divided into three regions along the perforant pathway (Brettschneider et al., 2013; Gómez-Pinedo et al., 2016; Takeda et al., 2009). TDP-43 pathology in the subiculum in ALS is rare; however, neuronal loss in this region is observed frequently and often precedes the pathological spread of TDP-43 to the DG (Brettschneider et al., 2013; Takeda et al., 2009). Moreover, an *in vivo* neuroimaging study reported the detection of subiculum complex atrophy during the early stages of ALS before progressive neurodegeneration was visible in other hippocampal subfields or subcortical structures (Westeneng et al., 2015). Recently, Gómez-Pinedo and colleagues showed that the amyloid cascade of the amyloid precursor protein is activated in the hippocampus of ALS patients (Gómez-Pinedo et al., 2016). These findings indicate that early and selective reduction in hippocampal subfield volumes (especially in the subiculum complex) in ALS patients is likely caused by a TDP-43 pathology-independent pathway and that TDP-43 pathology-related hippocampal alterations may emerge initially in the DG during the advanced stages of ALS.

Notably, neuroimaging studies of hippocampal volumes in patients with ALS have been largely contradictory to date, with studies reporting no changes, smaller volumes, or volumetric reductions that were restricted to specific hippocampal subfields in patients compared with healthy subjects (Christidi et al., 2019; Finegan et al., 2019; Machts et al., 2018; Tae et al., 2020; Westeneng et al., 2015). We hypothesise that these discrepancies are likely caused by volume alterations in different portions of the hippocampus that emerge at different disease stages. In particular, according to neuropathological studies, subiculum atrophy appears to emerge early and may represent a TDP-43 pathology-independent phase of hippocampal alteration, whereas DG atrophy is more likely to emerge during Braak stage 4, when TDP-43 spreads to the hippocampus (Westeneng et al., 2015; Takeda et al., 2009). Such pathologies may coexist in the hippocampus during the later stages of the disease (i.e., Braak stage 4) and therefore, hippocampal atrophy can vary across different disease stages in ALS patients.

Thus, it is necessary to investigate *in vivo,* the degree to which hippocampal subfield volumes differ between ALS patients at different stages. Therefore, in the present study, we used the well-validated King's clinical staging system for ALS to classify patients into different disease stages, according to previous studies that have shown greater homogeneity among ALS patients at the same disease stage (Roche et al., 2012). We investigated hippocampal atrophy patterns across subfields and anterior-posterior segments in ALS patients at different King's stages using *in vivo* structural MRI. The second aim of this study was to use the thalamus (representing Braak stages 2), corticostriatal tract and perforant pathway (DTI data were acquired from a subset of ALS patients; corticostriatal tract and perforant pathway involvement are thought to represent Braak stages 3 and 4, respectively) as structural controls to compare the sequence of alterations in these structures and hippocampal subfields. If subiculum alterations that occur prior to changes in other hippocampal subfields, thalamus and white matter tracts may hint the existence of a TDP-43 pathology-independent pathway in ALS patients.

2. Materials and methods

2.1. Participants

In this study, 76 patients newly diagnosed with ALS between November 2019 and November 2020 were included. All patients met the revised El Escorial criteria for possible, probable, or definite ALS (Brooks. et al., 2000). All patients presented with progressive disability during a 3-month telephone follow-up appointment. The exclusion criteria for ALS patients were as follows: 1) family history of ALS; 2) inability to complete an MRI scan; 3) frontotemporal dementia (FTD; because it is uncommon (prevalence of 4.7%) in Chinese patients with sporadic ALS; combined with FTD; 4) other severe neurological or psychiatric condition; and 5) refusal to participate. At our centre, the Neary criteria were used to diagnose FTD (Neary et al., 1998). In addition, we recruited 94 age-matched healthy controls (HCs). HCs were subjected to the same exclusion criteria as ALS patients.

2.2. Ethical approva

This study was approved by the research ethics committee of the School of Medicine, Shandong University. Participant information was collected after all patients and HCs were informed about the purpose of the study and provided informed written consent.

2.3. Clinical screening

We recorded demographic and clinical information of all patients, which included age, sex, education, family history of neurological disease, comorbid conditions, site of symptom onset and disease duration. The revised ALS Functional Rating Scale (ALSFRS-R) was used to assess disease severity (Cedarbaum et al., 1999). Depression and anxiety were assessed using the Hamilton Depression Rating Scale (HDRS) and Hamilton Anxiety Rating Scale (HARS), respectively (Liu et al., 2018). ALS patients underwent a neuropsychological test battery to screen for cognitive and behavioural features (Liu et al., 2018), which included the Mini-Mental State Examination (MMSE), Frontal Assessment Battery (FAB), Boston Naming Test (BNT) and Auditory Verbal Learning Test (AVLT). Behavioural symptoms were assessed via interview with the informant and quantified using the Frontal Behavioural Inventory (FBI).

2.4. ALS staging

During clinical screening, clinical staging was evaluated using the King's clinical staging system (Roche et al., 2012). Stages 1–3 of the disease are based on the body regions involved (bulbar, upper limbs and lower limbs), and Stage 4 is defined as the need for nutritional or respiratory support. The King's staging system might be closely linked to

anatomical spread. Because of the potentially problematic naming of Stage 4 milestones, ALS patients at stage 4 were less homogeneous compared with the other three stages. Moreover, only two patients were classified as King's stage 4; therefore we decided to exclude stage 4 from the final analysis.

2.5. MRI acquisition

All MRI data were obtained on a 3.0 T magnetic resonance system (Philips Medical System Ingenia scanner) with dStream head coil. Structural images of the whole brain were scanned using a three-dimensional (3D) fast spoiled gradient-echo sequence: repetition time (TR) = 6.7 ms, echo time (TE) = 3.0 ms, matrix = 68 × 68, voxel size = 1 mm × 1 mm × 1 mm, field of view (FOV) = 240 mm × 240 mm, slice thickness = 1.0 mm, no slice gap, and a total of 180 slices. For diffusion tensor imaging (DTI) analyses, we used a spin-echo sequence: TR = 4900 ms, TE = 95 ms, FOV = 224 mm × 224 mm, matrix = 112 × 112, b = 0 and 1000 s/mm2, slice thickness = 2 mm, no slice gap, voxel size = 2 mm × 2 mm × 2 mm, 32 gradient directions, and a total of 70 slices. FLAIR data were scanned using TR = 7000 ms, Flip Angle 90°, TE = 125 ms, acquisition matrix = 272 × 176, and slice thickness 6 mm. The MRI scan and clinical screening were performed within 3 days.

2.6. Postprocessing

2.6.1. Global hippocampus and thalamus volume

We used the thalamus, the corticostriatal tract, corticostriatal tract, and the proximal portion of perforant path as structural controls in this study. We compared the sequence of alterations that occur between these structures and hippocampal subfields to demonstrate the existence of co-pathologies in ALS patients. First, the whole hippocampus and thalamus volumes were automatically segmented and calculated using FreeSurfer version 7.1.1 (http://surfer.nmr.mgh.harvard.edu). The procedure, which including motion correction, intensity normalisation, automated topology corrections, and the automatic segmentation of cortical and subcortical regions, has been documented in detail elsewhere.[28] Subsequently, total intracranial volume (TIV) was calculated for each subject for further analysis as a covariate.

2.6.2. Tractography of the corticostriatal pathway and proximal portion of the perforant pathway

In the present study, a subset of ALS patients (n = 28) and HCs (n = 30) underwent DTI. Using the probabilistic tractography approach, the corticostriatal pathway (representing Braak stage 3) and the proximal portion of the perforant pathway (representing Braak stage 4) were determined using the procedure described in a previous study (Pan et al., 2020; Su et al., 2020). The seed region of interest had a radius of 5 mm, and the target region of interest had a radius of 10 mm. The coordinates of the seed and target regions are described in detail by Kassubek and colleagues (Kassubek et al., 2014).

2.6.3. Hippocampal subfield and anterior–posterior segment profiles

In the present study, the hippocampal subfields were automatically segmented and measured using a package available in FreeSurfer 7.1.1 (Iglesias et al., 2015). Using this algorithm, the hippocampus was accurately segmented into the following subfields: subiculum, presubiculum, parasubiculum, molecular layer, cornu ammonis (CA)1, CA2/3, CA4, hippocampal tail, hippocampal fissure, fimbria, hippocampus-amygdala transition area (HATA) and molecular and granule cell layer of the DG (GC-ML-DG) (Fig. 1). Moreover, automated rotation and segmentation procedures that are available in FreeSurfer 7.1.1 were used to reliably identify the hippocampus along its longitudinal axis. This procedure has been well-validated through comparisons with the gold standard (Lerma-Usabiaga et al., 2016). This method was used to further segment the hippocampal subfields of the subiculum, presubiculum, GC-ML-DG, molecular layer and CA1–4 into anterior and posterior portions.

2.7. Statistical analysis

2.7.1. Clinical data analysis

Continuous variables are reported as means and standard deviations, whereas categorical variables are reported as frequencies and proportions. Student's *t*-tests and analyses of variance (ANOVA) were used to compare continuous variables (with Mann-Whitney *U* tests as necessary). Categorical variables were compared using chi-squared tests. Post hoc tests were performed to identify pairwise group differences and corrected using Bonferroni correction. *P*-values < 0.05 indicated significance. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY).

2.7.2. MRI data analysis

For each grey matter (GM) structure and tractography measure, ANOVA models were constructed to investigate differences in structural volumes and interest-based fractional anisotropy (FA) values of tracts between groups. We used age, sex and TIV (for GM volumes) as covariates. Post hoc tests were performed to identify pairwise group differences using Bonferroni correction. A $p < 0.05$ was considered significant.

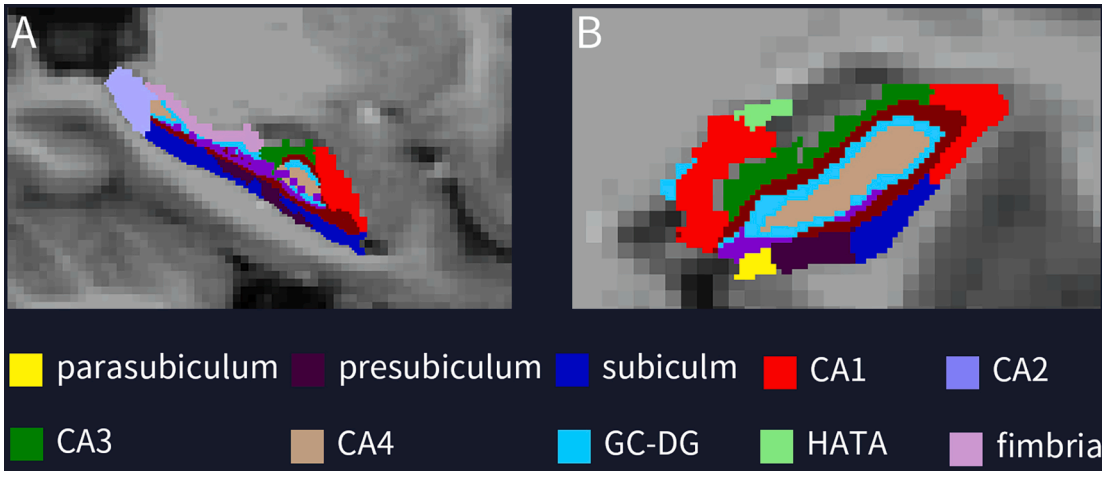

Fig. 1. Atlas-based segmentation of the hippocampus.

3. Results

3.1. Demographic and clinical information

Seventy-six ALS patients and 94 HCs were included in the present study. Cognitive tests could not be performed in two patients with ALS because of serious physical disability. Compared with HCs, ALS patients had lower MMSE and FAB scores ($p < 0.05$). Compared with HCs, ALS patients had higher HDRS and HARS scores ($p < 0.05$). Demographic and clinical information of the ALS patients and HCs are shown in Table 1.

3.2. Findings between patients with ALS at different King's clinical stages

Patients with ALS were divided into their corresponding King's clinical stage at clinical screening according to the body regions involved. No significant differences were found between the three patient subgroups except age and ALSFRS-R scores. Post hoc analyses showed that compared with ALS patients at King's stage 1, patients at King's stages 3 were significantly older. Moreover, ALSFRS-R scores were significantly different between King's stage groups. Demographic and clinical information of each King's stage group are shown in Table 2.

3.3. Global hippocampus and thalamus volumes

Compared with HCs, ALS patients had significantly more atrophy in the left and right global hippocampal volumes; however, no significant differences were observed in global thalamus volumes (Table 1). Significant differences in global hippocampus and thalamus volumes were found between King's stage patient subgroups and HCs. Post hoc analyses showed that compared with HCs, ALS patients at King's stage 2 had significantly lower left hippocampal volumes, and ALS patients at King's stage 3 had significantly lower bilateral hippocampal volumes. Profiles of global hippocampus volumes for HCs and ALS patients at each disease stage are presented in Fig. 2. There were no significant differences in hippocampus volumes between the three King's stage patient subgroups. Compared with HCs and ALS patients at King's stages 1 and 2, patients at King's stage 3 had significantly greater atrophy of the bilateral thalamus. Profiles of global thalamus volumes for HCs and ALS patients at each disease stage are presented in Fig. 3.Fig. 4.

Table 1
Demographic and clinical features of ALS patients and HCs.

	ALS patients (n = 76)	HCs (n = 94)	P-value
Age (years)	57.5 ± 11.3	55.2 ± 6.8	0.11
Men/Women (n)	43/33	35/59	0.01
Education	9.5 ± 4.0	10.3 ± 3.8	0.17
ALS duration (mo)	11.6 ± 7.5	–	–
Bulbar ALS onset n, (%)	17 (22.3)	–	–
ALSFRS-R score	41.4 ± 3.2	–	–
Riluzole, n (%)	5 (6.5)	–	–
King's clinical stage (stages 1/2/3), %	26.3%, 52.6%, 21.0%	–	–
MMSE	26.6 ± 3.0	28.3 ± 1.9	< 0.01
FAB	14.6 ± 2.0	17.2 ± 0.7	< 0.01
FBI	1.2 ± 1.4	–	–
BNT	23.7 ± 4.2	24.9 ± 4.1	0.16
AVLT, short delayed (5 min)	7.2 ± 3.1	7.7 ± 3.3	0.24
AVLT, long delayed (20 min)	6.5 ± 3.3	7.4 ± 2.8	0.06
HARS	8.1 ± 4.6	2.6 ± 3.7	< 0.01
HDRS	11.4 ± 7.1	3.4 ± 3.8	< 0.01
Global thalamus, left	6412.6 ± 872.5	6631.8 ± 713.8	0.07
Global thalamus, right	6180.6 ± 782.8	6356.4 ± 622.1	0.15
Global hippocampus, left	3521.5 ± 427.0	3792.5 ± 333.7	< 0.05*
Global hippocampus, right	3694.0 ± 501.8	3927.8 ± 334.9	< 0.05*

ALS: amyotrophic lateral sclerosis; HC: healthy control; ALFRS-R: ALS Functional Rating Scale-Revised; MMSE: Mini-Mental State Examination; FAB: Frontal Assessment Battery; FBI: Frontal Behavioral Inventory; BNT: Boston Naming Test; AVLT: Auditory Verbal Learning Test; HARS: Hamilton Anxiety Rating Scale; HDRS: Hamilton Depression Rating Scale. Volume (mm^3). * after Bonferroni correction.

Table 2
Demographic and clinical information of each King's stage subgroup.

	Stage 1 (n = 20)	Stage 2 (n = 40)	Stage 3 (n = 16)	F or χ^2	P-value
Age (years)	53.7 ± 12.3	57.0 ± 10.8	63.2 ± 9.5	3.45	0.04
Men/Women (n)	11/9	25/15	7/9	1.66	0.43
Education	10.3 ± 3.9	9.1 ± 4.1	9.5 ± 3.6	0.53	0.59
ALS duration (mo)	9.1 ± 4.1	12.1 ± 8.5	13.1 ± 7.6	1.55	0.22
Bulbar ALS onset n, (%)	5 (25)	9 (22.5)	3 (18.7)	0.07	0.96
ALSFRS-R score	44.5 ± 1.4	41.3 ± 2.3	37.8 ± 2.9	38.05	< 0.01
MMSE	27.4 ± 2.6	26.4 ± 2.6	26.1 ± 4.1	1.02	0.36
FAB	15.1 ± 1.1	14.7 ± 2.1	13.8 ± 2.4	1.85	0.17
BNT	25.2 ± 4.8	23.3 ± 3.8	22.8 ± 4.1	1.84	0.16
AVLT, short delayed	7.9 ± 3.2	7.0 ± 2.9	6.8 ± 3.2	0.68	0.51
AVLT, long delayed	7.2 ± 3.5	6.2 ± 3.3	6.1 ± 3.2	0.67	0.50
HARS	8.7 ± 4.8	7.6 ± 4.6	8.7 ± 4.0	0.44	0.64
HDRS	12.4 ± 9.8	10.5 ± 5.9	12.3 ± 5.4	0.63	0.53

ALS: amyotrophic lateral sclerosis; ALFRS-R: ALS Functional Rating Scale-Revised; MMSE: Mini-Mental State Examination; FAB: Frontal Assessment Battery; FBI: Frontal Behavioral Inventory; BNT: Boston Naming Test; AVLT: Auditory Verbal Learning Test; HARS: Hamilton Anxiety Rating Scale; HDRS: Hamilton Depression Rating Scale.

3.4. Tractography of the corticostriatal pathway and the proximal portion of the perforant pathway

Neither the corticostriatal tract nor the proximal portion of the perforant pathway showed significant differences in FA values between ALS patients and HCs.

3.5. Profiles of hippocampal subfields and anterior-posterior segments of the longitudinal axis

Significant differences between King's stage patient subgroups and HCs were found for the bilateral posterior subiculum, bilateral anterior subiculum, bilateral posterior presubiculum, bilateral anterior presubiculum, right parasubiculum, bilateral anterior CA1, bilateral anterior CA2/3, bilateral anterior CA4, left posterior CA4, bilateral fimbria, bilateral hippocampal tail, right hippocampal fissure, bilateral HATA, bilateral anterior molecular layer, bilateral posterior molecular layer, bilateral posterior GC-ML-DG, bilateral anterior GC-ML-DG, bilateral anterior hippocampus and bilateral posterior hippocampus (Supplemental Table 1).

Post hoc analyses showed that compared with HCs, ALS patients at King's stage 1 had lower volumes in the left posterior subiculum ($P = 0.015$), right posterior subiculum ($P = 0.003$) and left posterior presubiculum ($p = 0.041$), but no differences were observed for any of the other subfields or global hippocampal volume. Compared with HCs, ALS patients at King's stage 2 had lower volumes in the left posterior subiculum ($p = 0.005$), right posterior subiculum ($p = 0.002$), left anterior subiculum ($p = 0.020$), left anterior presubiculum ($p = 0.009$), right anterior presubiculum ($p = 0.035$), left anterior CA1 ($p = 0.018$), left anterior molecular layer ($p = 0.002$), right anterior molecular layer ($p = 0.005$), left posterior molecular layer ($p = 0.039$), left HATA ($p = 0.001$), left hippocampal tail ($p = 0.004$), right hippocampal tail ($p = 0.022$),

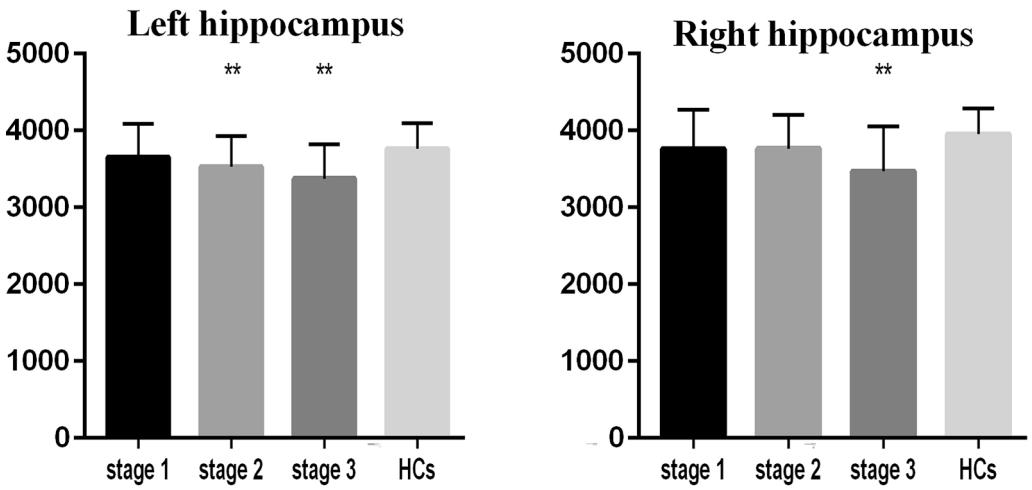

Fig. 2. Global hippocampal profiles for ALS patients at each disease stage and HCs.

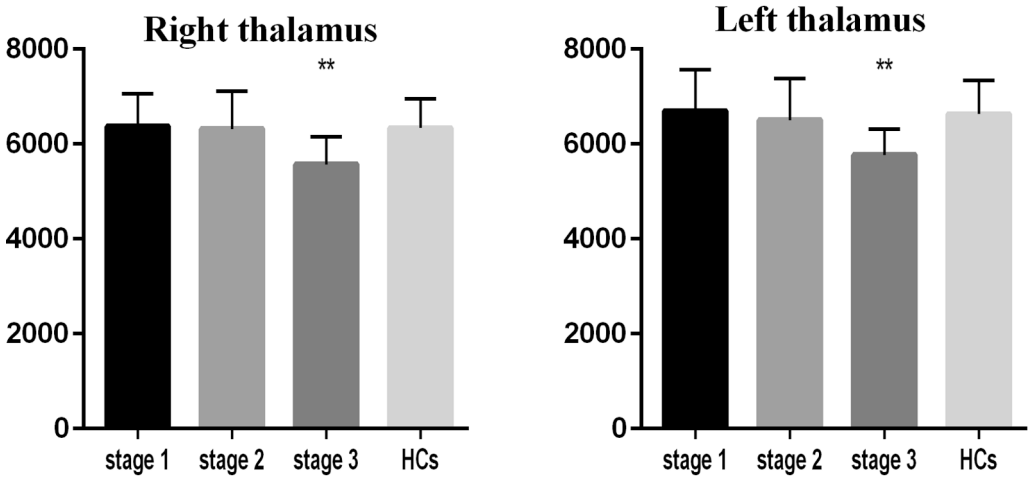

Fig. 3. Global thalamic profiles for ALS patients at each disease stage and HCs.

right fimbria ($p = 0.019$), left hippocampus ($p = 0.002$) and right hippocampus ($p = 0.016$). Compared with HCs, ALS patients at King's stage 3 had lower volumes in the left posterior subiculum ($p < 0.001$), right anterior subiculum ($p = 0.005$), right posterior subiculum ($p < 0.001$), right posterior presubiculum ($p = 0.001$), right anterior presubiculum ($p = 0.021$), left anterior molecular layer ($p = 0.001$), right anterior molecular layer ($p = 0.001$), left posterior molecular layer ($p = 0.005$), right posterior molecular layer ($p = 0.001$), left posterior GC-ML-DG ($p < 0.001$), right posterior GC-ML-DG ($p = 0.011$), left anterior GC-ML-DG ($p = 0.001$), right anterior GC-ML-DG ($p = 0.001$), left anterior CA1 ($p = 0.001$), right anterior CA1 ($p = 0.001$), left anterior CA4 ($p = 0.001$), right anterior CA4 ($p = 0.001$), left posterior CA4 ($p = 0.003$), left fimbria ($p = 0.014$), right fimbria ($p = 0.001$), left anterior CA2/3 ($p = 0.002$), right anterior CA2/3 ($p = 0.001$), left HATA ($p = 0.003$), right HATA ($p = 0.001$), left hippocampus ($p = 0.001$) and right hippocampus ($p = 0.001$). Compared with ALS patients at King's stage 1, patients at King's stage 3 had lower left anterior CA2/3 ($p = 0.036$) and left anterior CA4 ($p = 0.048$) volumes. Compared with ALS patients at King's stage 2, patients at King's stage 3 had lower volumes in the right anterior CA2/3 ($p = 0.026$), right anterior CA4 ($p = 0.019$), right anterior GC-ML-DG ($p = 0.037$) and right anterior molecular layer ($p = 0.034$). Profiles of the left and right hippocampal subfields and anterior-posterior portions of the longitudinal axis for HCs and ALS patients at each King's stage are presented in Figs. 4 and 5, respectively.

4. Discussion

In a relatively large cohort of ALS patients, using a state-of-the-art hippocampal segmentation approach, we observed varied patterns of hippocampal atrophy in ALS patients across different King's clinical disease stages. Compared with HCs, ALS patients at King's stage 1 had lower volumes in the bilateral posterior subiculum and presubiculum, whereas other subfields and global hippocampal volume remained unaffected. ALS patients at King's stage 2 exhibited lower volumes in the bilateral posterior subiculum, left anterior presubiculum and left global hippocampus. ALS patients at King's stage 3 showed prominently lower volumes in the bilateral posterior subiculum, GC-ML-DG and global hippocampus. Compared with ALS patients at King's stage 1, ALS patients at King's stage 3 had lower left anterior CA2/3 and CA4 volumes. Compared with ALS patients at King's stage 2, ALS patients at King's stage 3 had lower volumes in the right anterior CA2/3, right anterior CA4, right anterior GC-ML-DG and right anterior molecular layer. Moreover, thalamic atrophy did not emerge until King's stage 3, and the perforant pathway remained normal in a subset of ALS patients. These findings suggest that patients with ALS may present a TDP-43 pathology-independent pathway additional pathologies that co-exist with TDP-43 pathology, which might exert an earlier and preferential effect on the subiculum complex. However, this viewpoint needs to be confirmed by further studies.

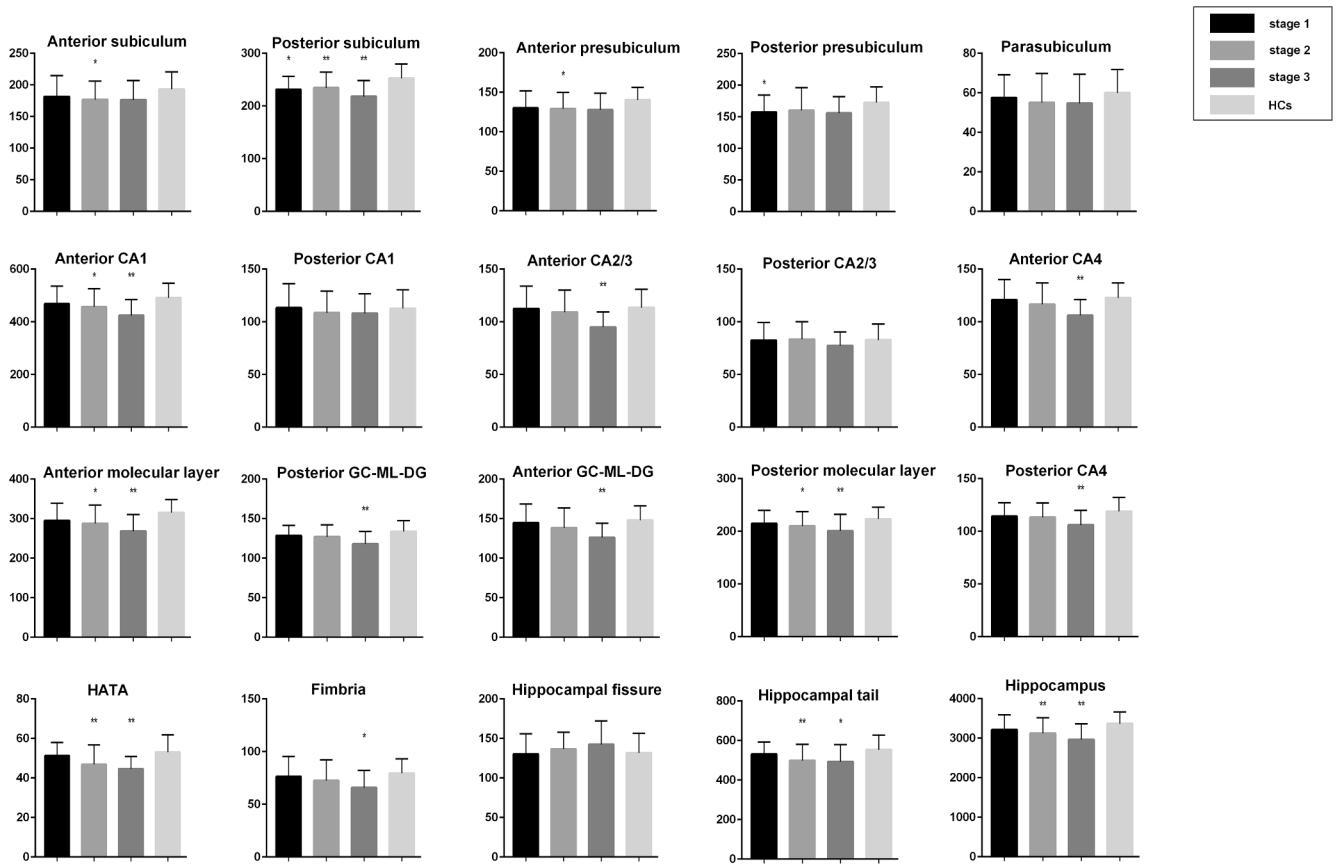

Fig. 4. Left hippocampal subfield and anterior-posterior portions of longitudinal axis profiles for ALS patients at each disease stage and HCs.

Numerous neuroimaging studies have been conducted to examine the incidence of hippocampal atrophy in ALS patients; however, these studies have generated inconsistent results (Christidi et al., 2019; Finegan et al., 2019; Machts et al., 2018; Tae et al., 2020; Westeneng et al., 2015). Some studies have reported no changes, while others have reported smaller volumes, and still others have reported focal atrophy limited to specific hippocampal subfields. To explore the extent of hippocampal subfield involvement in patients with ALS, in a longitudinal study, Westeneng et al. recruited a total of 112 patients and 60 HCs, and 39 patients underwent a follow-up scan after 5.5 months. Similar to our study, at baseline, they found significantly smaller left presubiculum in patients with ALS, and atrophy of the CA4/DG could be detected at the follow-up scan (Westeneng et al., 2015). To characterize the mesial temporal lobe profile of patients with AD, patients with ALS, and HCs, Christidi et al. recruited 50 patients with ALS, 18 patients with AD, and 40 HCs in a cross-sectional study. In contrast to Westeneng et al., Christidi et al. reported the CA 2/3 subfield and the HATA are the most affected regions in patients with ALS, where the presubiculum and subiculum are the most vulnerable regions in AD patients (Christidi et al., 2019). Recently, Finegan et al. examined hippocampal subfields atrophy in 100 ALS patients in comparison to 117 HCs. They reported focal atrophy can be detected in patients with ALS in the DG, molecular layer, and CA4 subfields compared to HCs, whereas the other hippocampal subfields remained unaffected (Finegan et al., 2019).

The controversy among these results from previous studies might largely result from the pattern of hippocampal atrophy in ALS differs at different disease stages. Consistent with the pattern observed in our patients with ALS at King's Stage 1, recently, Tae et al. reported that global hippocampal volumes did not differ significantly between ALS patients and HCs in a group of relatively early-phase ALS patients, as the disease duration of the patients in their study was only 12.99 months. However, they did not analyse hippocampal subfield volumes (Tae et al., 2020). Consistent with the pattern observed in our patients with ALS at King's Stage 2, Westeneng and colleagues reported that ALS patients had reduced presubiculum and global hippocampal volumes and that the subiculum volume tended to be reduced (Westeneng et al., 2015). Indeed, in accordance with the neuropathological studies, which report the detection of DG atrophy during advanced phases of ALS, DG atrophy emerged in patients with ALS at King's Stage 3 in the present study (Braak et al., 2013). In line with our study, Finegan and colleagues reported prominent DG degeneration in a group of advanced-stage ALS patients (with mean ALSFRS-R scores of 36.6) (Finegan et al., 2019). Unfortunately, none of these studies used the well-validated King's clinical staging system to divide ALS patients into homologous subgroups at different stages.

According to Braak, and hippocampal pathological stages described by Takeda and colleagues, TDP-43 pathology-related hippocampal alterations emerge initially in the DG (through the perforant pathway) in patients with ALS, which may be a feature of end-stage ALS (Braak et al. 2013; Takeda et al., 2009). However, previous neuroimaging studies in ALS patients have indicated that *in vivo* global hippocampal atrophy could be detected at early stages of ALS, and hippocampus volumes have been shown to associate with episodic memory function (Machts et al., 2021; Westeneng et al., 2015). In the present study, we demonstrated that the earliest detectible hippocampal alterations in ALS patients occurred in the posterior subiculum and presubiculum, and these alterations emerged at King's stage 1, which was earlier than the alterations in the global hippocampus (at King's stage 2), DG, thalamus (at King's stage 3) or perforant pathway. This indicates that subiculum atrophy occurs earlier and independent of TDP-43 pathology in ALS (Braak et al. 2013; Takeda et al., 2009). Taken together, our data and those of others suggest that patients with ALS have additional pathology that is independent of TDP-43 pathology, which exerts an earlier and preferential effect on the subiculum complex (Brettschneider et al.,

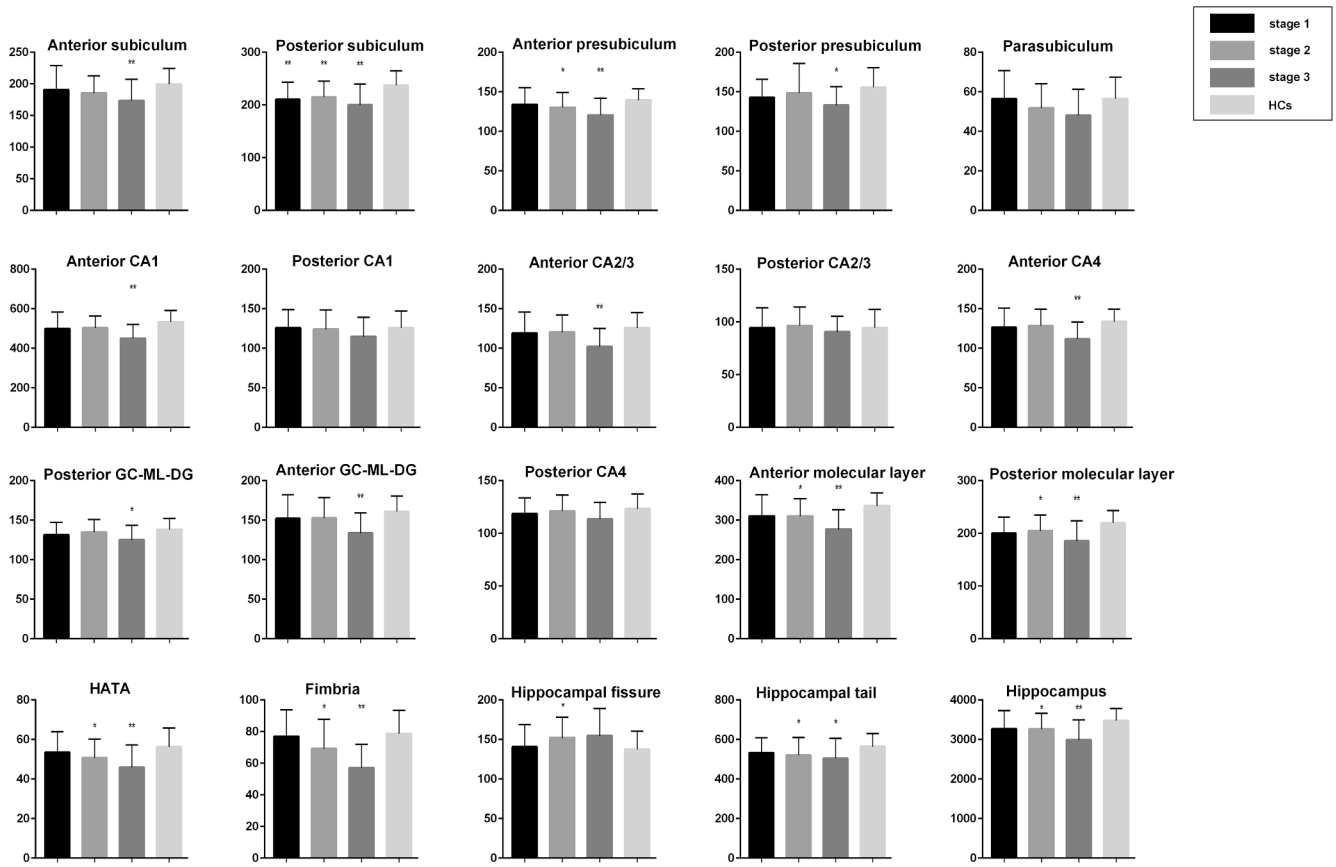

Fig. 5. Right hippocampal subfield and anterior-posterior portions of longitudinal axis profiles for ALS patients at each disease stage and HCs.

2013; Gómez-Pinedo et al., 2016; Takeda et al., 2009).

As observed in other neurodegenerative conditions, mixed pathologies also exist in ALS (Josephs et al., 2014; Hamilton et al., 2004; Jucke et al., 2018; Yokota et al., 2020). Increasingly, studies have shown that at least 20% of ALS patients present significant AD pathology of both Aβ and tau (Brettschneider et al., 2012; Hamilton et al., 2004). Previous studies have demonstrated that AD pathology induces preferential vulnerability of the subiculum (particularly the posterior subiculum) along the AD continuum (Carlesimo et al., 2015; de Flores et al., 2020). Robinson and colleagues showed that in ALS patients, AD pathology is primarily localised to the CA1 and subiculum subfields of the hippocampus (Robinson et al., 2014). Additionally, hippocampal tau pathology has been shown to associate with MMSE scores in ALS patients (Brettschneider et al., 2012; Takeda et al., 2009). Recently, Gómez-Pinedo and colleagues showed that in ALS patients, the amyloid cascade of the amyloid precursor protein is activated in the hippocampus of ALS patients, and cytoplasmic Aβ peptide and pTDP-43 expression levels are moderately correlated, which is further evidence that mixed pathologies exert synergistic effects in the hippocampus (Gómez-Pinedo et al., 2016). Therefore, in similar fashion to TDP-43 pathology playing a role in AD, we suggest that AD pathology also plays a role in ALS, which affects the subiculum at the early stage of ALS (Josephs et al., 2014). In contrast to the TDP-43/tau hippocampal co-localisations and synergy that occur at the early stage of AD, AD pathology in ALS may exhibit a distinct spatiotemporal pattern (Josephs et al., 2014). The motor cortex and subiculum seem to represent two independent centres of ALS during the early stages of the disease, which represent TDP-43 pathology and AD pathology, respectively, and these pathologies may converge as the disease progresses toward advanced stages.

Importantly, if our findings are confirmed in further studies, they will have a profound effect on the understanding of the aetiology and pathogenic mechanisms underlying ALS and other neurodegenerative diseases. These neurodegenerative diseases may be associated with specific genetic modifiers but share common environmental risk factors, such as age, trauma and oxidative stress, which may partially underlie the co-pathology observed in these conditions (Chornenkyy et al., 2019; Schmidt et al., 2010). Indeed, ALS patients often lack a strong family history of the disease, and those with TARDBP mutations are rare; although the APOE ε4 allele, which is strongly associated with the deposition of the Aβ peptide and AD pathogenesis, has also been associated with TDP-43 proteinopathy (Schmidt et al., 2010). Depending on the expression of mixed brain pathologies, different stages of ALS may not lie along the same continuum, which appears to be especially true when the dynamic processes of co-pathology in the hippocampus are considered. Thus, we suggest that the classification of ALS patients into homologous subgroups may be necessary for clinical trials and that hippocampal subfield volumes and the King's clinical staging system may provide a valid and meaningful approach (Roche et al., 2012).

The present study has several limitations. First, we used a cross-sectional design, which did not allow us to determine causality between different pathologies and hippocampal subfields alterations. Second, our results were susceptible to selection bias because most ALS patients who visit our centre (the largest ALS centre in the Shandong province) have a relatively short disease course. Third, we only used the MMSE, BNT, AVLT and FAB to screen cognitive functions. Fourth, although we used age, sex and TIV as covariates, patients and HCs were not sex matched. In the present study, all HCs were continuously recruited from the community. Recently, Lv et al. explored whether cognitive impairment was associated with mortality among community-dwelling older Chinese people and found that 44.9% (5264/11732) of subjects were men. Fifth, DTI data were available for only a subset of ALS patients and could not be performed for clinical staging in these patients. Therefore, our findings require confirmation in future studies. Finally, we did not perform genetic testing. However, the ALS patients

included in this study were sporadic cases, and very few sporadic ALS patients in China carry genetic mutations (Liu et al., 2016).

5. Conclusions

Our study provides a comprehensive profile of hippocampal alterations in ALS patients. Our findings suggest that the pattern of hippocampal atrophy in ALS patients differs significantly between patients at different King's clinical disease stages. Future studies in ALS patients that focus on the hippocampus are needed to further understand the etiology and pathogenic mechanisms underlying ALS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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