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Hippocampal degeneration in patients with amyotrophic lateral sclerosis

Susanne Abdulla [a,b,c,*], Judith Machts [c], Jörn Kaufmann [a], Karina Patrick [a], Katja Kollewe [b], Reinhard Dengler [b], Hans-Jochen Heinze [a,c,d], Susanne Petri [b], Stefan Vielhaber [a,c], Peter J. Nestor [c]

[a] Department of Neurology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
[b] Department of Neurology, Hannover Medical School, Hannover, Germany
[c] German Center for Neurodegenerative Diseases, Magdeburg, Germany
[d] Leibniz Institute for Neurobiology, Magdeburg, Germany

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ABSTRACT

There is increasing appreciation of non-motor system involvement in amyotrophic lateral sclerosis (ALS), although its full extent and clinical significance remains to be established. This study tested the hypothesis that memory impairment in patients with ALS is related to hippocampal degeneration. Consecutive patients with ALS (58) and 29 matched controls participated in standardized neuropsychological assessment and magnetic resonance imaging. Patients with ALS performed worse in global cognitive functioning and executive and verbal memory tests ($p < 0.05$). The hippocampus was manually segmented in each hemisphere, and volumes were calculated with correction for intracranial volume. Analysis of covariance, controlled for the effect of age and education years, showed significantly smaller hippocampal volume on the right ($p = 0.004$) in patients with ALS. Verbal memory test performance correlated with the left hippocampal volume in patients with ALS ($p < 0.05$), although there was no significant correlation with tests of executive function and clinical variables underscoring the specificity of the present findings. Hippocampal volume loss and its correlation with the severity of verbal memory impairment highlight significant hippocampal involvement which can occur as a non-motor deficit in patients with ALS.

1. Introduction

In patients with amyotrophic lateral sclerosis (ALS), increasing evidence shows that neurodegeneration not only affects the motor system but also involves extra-motor brain regions and can result in cognitive and behavioral impairment. Nearly 50% of patients with ALS show some degree of cognitive impairment and up to 14% suffer from frontotemporal dementia (Phukan et al., 2011).

Several recent studies focused on the clinical and genetic overlap between frontotemporal dementia and ALS (Cooper-Knock et al., 2012; Hsiung et al., 2012; Stewart et al., 2012), but less attention has been paid to cognitive abilities more generally. The most consistently documented neuropsychological deficits are executive dysfunction, verbal fluency, and behavioral changes as well as neuropsychiatric symptoms although there has been some less consistent evidence of memory impairment (Abrahams et al., 2005; Christidi et al., 2012; Goldstein and Abrahams, 2013; Mioshi et al., 2014). In fact, with the emphasis on executive deficits in patients with ALS, memory has been somewhat neglected; some studies have reported neuropsychological changes regarding memory performance (Jelsone-Swain et al., 2012; Münte et al., 1998; Schreiber et al., 2005), but no studies specifically addressed whether memory in patients with ALS is an outcome of the hippocampal involvement. Memory encoding is a core function of the hippocampus. For instance, research in epilepsy surgery on hippocampal lesions and neuropsychological deficits has shown that verbal learning and memory correlated inversely with the extent of the left hippocampal resection whereas larger resections of the right hippocampus resulted in poorer figural memory performance (Helmstaedter et al., 2011; Jones, 1974; Jones-Gotman and Milner, 1978).

In patients with ALS, there is evidence of hippocampal involvement from neuropathologic and neuroimaging studies (Bede et al., 2013; Brettschneider et al., 2012; Takeda et al., 2007,

* Corresponding author at: Department of Neurology, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany. Tel.: +49 511 5322391; fax: +49 511 5323115.
E-mail address: Abdulla.Susanne@mh-hannover.de (S. Abdulla).

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2009). Neuropathologic changes include presynaptic degeneration of the outer molecular layer of the dentate gyrus and TDP-43 positive inclusions (Brettschneider et al., 2013; Phukan et al., 2011; Takeda et al., 2009). Hippocampal lesions in patients with ALS are relatively disease specific as the outer molecular layer of the dentate gyrus is less susceptible to degeneration in patients with Alzheimer's disease (Takeda et al., 2009). Neuropathologic studies suggested that memory deficits are partially associated with the hippocampal pathology in ALS (Brettschneider et al., 2012; Takeda et al., 2007, 2009). Typically long lag times between cognitive and pathologic assessments in clinicopathological studies, however, limit conclusions about the clinical significance of degeneration during life. Recently, 1 neuroimaging study, which demonstrated extensive basal ganglia pathology in ALS, provided evidence for hippocampal degeneration in both C9orf72-negative and C9orf72-positive patients with ALS (Bede et al., 2013). Whether there is any relationship between in vivo hippocampal volumetry and neuropsychological performance in ALS, however, remains unclear. The aim of this study was, therefore, to assess the frequency and magnitude of hippocampal atrophy in patients with ALS and whether such changes have any impact on memory performance.

2. Methods

2.1. Participants

The study was approved by the institutional ethics committee of Otto von Guericke University, Magdeburg, and all patients provided written informed consent before enrollment. Between April 2011 and March 2012, a total of 60 consecutive patients with possible, probable, or definite ALS according to the revised El Escorial Criteria (Brooks et al., 2000), and 30 healthy controls were initially recruited into the study. Two patients were excluded from the analysis because they did not undergo neuropsychological assessment, and 1 healthy control was excluded because neuropsychological performance was significantly impaired leaving n = 58 patients with ALS and n = 29 controls in the analyses. At the same visit, each participant underwent a clinical examination, a comprehensive neuropsychological test battery, and was graded using the revised ALS functional rating scale (Cedarbaum et al., 1999). The site of onset of the first symptom was classified as bulbar or limb. In addition, blood samples from patients were screened for mutations in C9orf72, ubiquilin-2, and SOD1 genes. Four patients fulfilled the revised criteria for behavioral variant frontotemporal dementia (bvFTD) (Rascovsky et al., 2011), one of which carried the C9orf72 mutation.

2.2. Neuropsychological assessment

The test battery comprised a range of standard German neuropsychological instruments for which normative data for the German population are available. Their scope was particularly informed by previous literature that suggested early involvement of executive functions in ALS (Strong et al., 2009). Global cognitive functioning was tested using the Montreal Cognitive Assessment (Nasreddine et al., 2005). Executive function was tested with the Regensburger verbal fluency test that comprises phonemic verbal fluency (letter "K") and flexibility (alternation of words with letter "G" and "R") (Aschenbrenner et al., 2000). Additionally, cognitive flexibility was tested with the Trail Making Test (ratio between part B and A, to adjust for motor impairment) (Reitan, 1958) and verbal working memory performance was evaluated with digit span forward and backward from the Wechsler Memory Scale-revised (Wechsler, 1987). To assess memory, the German adaption of the Rey Auditory Verbal Learning Test (RAVLT) was applied (Schmidt, 1996) using the following parameters for analysis: learning (total sum of trials 1 through 5), immediate recall (total number of words recalled after interference), delayed recall (total number of words recalled after 20–30 minutes delay) and recognition (total number of words recognized from list A corrected for false positive and interference words). Visuoconstructive ability was tested using the Rey Complex Figure Test (copy) (Osterrieth, 1944). Mood was assessed using the Beck Depression Inventory-II (Beck et al., 1996).

2.3. Magnetic resonance imaging acquisition

High-resolution, T1-weighted 3-dimensional structural magnetic resonance imaging scans of the brain were acquired on a 3T Siemens VERIO Magnetom scanner (Siemens, Erlangen/Germany) with a 32-channel head coil using a 3D-MPRAGE (Mugler and Brookeman, 1990) sequence (echo time = 4.82 ms, repetition time = 2500 ms, inversion time = 1100 ms, flip angle = 7°, isotropic voxel size = 1.0 mm^3). Each subject's brain was acquired in stereotaxic alignment by matching the axial plane to the anterior commissure-posterior commissure line and the sagittal plane to the interhemispheric fissure.

2.4. Hippocampal volumetry

The contours of the hippocampus were traced on raw images using the software MultiTracer version 1 (Woods, 2003, http://www.bmap.ucla.edu/portfolio/software/MultiTracer/) by an investigator (Susanne Abdulla) blinded to diagnostic group. Intra-rater reliability and inter-rater reliability (segmented by a second investigator) in hippocampal volumetry were assessed by segmentation of the left and the right hippocampus in 10 randomly selected MRI scans of patients and healthy controls of the present study cohort. Intraclass correlation coefficient was $r = 0.91$ for intra-rater reliability and $r = 0.89$ for inter-rater reliability. For delineation, MultiTracer was set to the most accurate interpolation method (FFT/Chirp-z). The neuroanatomic criteria for segmentation of the hippocampus were based on the recently developed protocol by Wisse et al. (2012) referencing the atlas of the human hippocampus by Duvernoy (2005). The border of the hippocampus was traced from rostral to caudal in magnified images (8×) of the coronal slices while simultaneously visualizing the sagittal orientation (magnification 5×). The axial view (magnification 5×) was also checked to ensure accurate tracing. Delineation was performed using freehand spline drawing technique, which is considered to offer higher precision than the previously used voxel-by-voxel approaches (Wisse et al., 2012). Hippocampal outlines included the hippocampus proper, subiculum, and dentate gyrus, whereas white matter of the alveus and fimbria, having high signal intensity, were excluded (Wisse et al., 2012). Non-hippocampal areas were surrounded with the same label and thus excluded from delineation. This applied to the invagination of the hippocampal sulcus and hippocampal sulcus residual cavities, which are enlarged spaces of cerebrospinal fluid typically located in the lateral portion of the hippocampus (Li et al., 2006). An example of hippocampal segmentation is displayed in Supplementary Fig. 1.

The classic volume was calculated by summing the areas for each plane of the outlined hippocampal gray matter multiplied by the slice thickness.

2.5. Total intracranial volume measurement

Total intracranial volume (TIV), gray matter and white matter volumes were assessed after classifying the 3D T1-weighted images into gray matter, white matter, and cerebrospinal fluid using the Gaussian mixture model within the unified segmentation approach (Ashburner and Friston, 2005) in SPM8 (Wellcome Trust Centre for