Title: Structural and functional hippocampal alterations in Multiple sclerosis and Neuromyelitis optical spectrum disorder

Abstract

Background: Hippocampal involvement may differ between multiple sclerosis (MS) and neuromyelitis optical spectrum disorder (NMOSD).

Objective: To investigate the morphometric, diffusion and functional alterations in hippocampus in MS and NMOSD and the clinical significance**.**

Methods: A total of 752 participants including 236 MS, 236 NMOSD and 280 healthy controls (HC) were included in this retrospective multi-center study. The hippocampus and subfield volumes, fractional anisotropy (FA) and mean diffusivity (MD), amplitude of low frequency fluctuation (ALFF) and degree centrality (DC) were analyzed, and their associations with clinical variables were investigated.

Results: The hippocampus showed significantly lower volume, FA and greater MD in MS compared to NMOSD and HC ($P<0.05$), while no abnormal ALFF or DC was identified in any group. Hippocampal subfields were affected in both diseases, though subiculum, presubiculum and fimbria showed significantly lower volume only in MS ($P<0.05$). Significant correlations between diffusion alterations, several subfield volumes and clinical variables were observed in both diseases, especially in MS ($R=-0.444\sim0.498$, $P<0.05$). FA and MD showed fair discriminative power between MS and HC, NMOSD and HC ($AUC>0.7$).

Conclusions: Hippocampal atrophy and diffusion abnormalities were identified in MS and NMOSD, partly explaining how clinical disability and cognitive impairment are differentially affected.

Manuscript details

Number of abstract words: 199 words

Number of manuscript words: 2997 words

Number of references: 35

Number of tables: 1

Number of figures: 4

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by demyelination and neurodegeneration[1], while neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory disease with astrocyte injury[2]. Cognitive impairment is a common feature of MS and NMOSD with a prevalence of approximately 40% to 70% in MS[3] and 54% to 57% in NMOSD[4].

The hippocampus is a complex structure composed of various subfields, which plays a crucial role in cognitive performance failure in MS and NMOSD[5, 6]. Hippocampal subfields have distinct anatomical and functional features and collectively contribute to standard cognitive processing. Studies of hippocampal subfields are scarce in MS and NMOSD, although understanding the complex mechanisms of hippocampus subfields is essential for developing imaging biomarkers for disease progression, particularly cognitive impairment[7]. Previous studies have reported that hippocampal subfields changes occurred even in early stage of MS[8, 9], and have identified the association between hippocampal subfield (e.g. dentate gyrus) alteration and impaired word learning in MS[10, 11].

Most previous studies focused on a single imaging modality[7, 12] or based on small-sized cohorts[3, 8]. Multi-modal magnetic resonance imaging (MRI) studies with large sample size, especially with a multi-center design, are needed to understand better the clinical significance of abnormal

hippocampal structure and function in MS and NMOSD.

We hypothesized that the hippocampus exhibits different patterns of involvement in MS and NMOSD. Therefore, we investigated the morphometric, diffusion and functional alterations in the hippocampus in MS and NMOSD, and assessed the correlations between hippocampal measurements and clinical variables, particularly cognitive scores, in a large multi-center cohort.

Materials and methods

Participants

A total of 863 participants (262 MS, 270 NMOSD and 331 healthy controls [HC]), aged between 16 and 65 years, were considered for this retrospective multi-center study in China carried out in seven centers between January 2009 and September 2019. Table e-1 listed the number of subjects recruited in each center. Informed consents from all participants have been obtained. This study was approved by local institutional review board. All subjects recruited fulfilled the inclusion criteria: (1) 2017 revised McDonald criteria for MS and 2015 criteria for NMOSD; (2) Relapsing-Remitting MS; (3) right-handedness; (4) either in relapsing phase (less than 4 weeks from the last relapse) or stable phase (more than 4 weeks from the last relapse). Clinical variables were collected, including expanded disability status scale (EDSS) scores, disease duration, anti-APQ4 serostatus of 163 NMOSD patients and the number of relapses. We excluded subjects based on the following criteria: (1) incomplete clinical assessment; (2) poor image quality; (3) a history of other neurological or neuropsychological diseases. After exclusion, 752 participants (236 MS, 236 NMOSD and 280 HC) were finally included in the current study.

A total of 161 subjects received the California Verbal Learning Test (CVLT)–Second Edition[13], 176 subjects received the Brief Visuospatial Memory Test (BVMT)–Revised[14], and 274 subjects received the Paced Auditory Serial

Addition Test (PASAT) measurements (3 seconds)[15] in this study. Figure 1 shows the flow chart of subjects included in this study.

MRI acquisition

MRI was acquired on 3.0T scanners, including T2 weighted (T2w), FLAIR (fluid attenuated inversion recovery) images, high-resolution 3D T1 weighted (T1w), diffusion tensor imaging and resting-state fMRI (functional MRI). The MRI scan parameters for each center are summarized in Table e-2. Among 752 participants, all subjects had T2w or FLAIR images, 721 subjects (216 MS, 227 NMOSD, 278 HC) had 3D T1w images, 709 subjects (233 MS, 222 NMOSD, 254 HC) had diffusion images, 636 subjects (208 MS, 200 NMOSD, and 228 HC) had resting-state fMRI images, and 583 subjects had complete four modalities images.

Lesion measurement

Based on T2w or FLAIR images, lesion masks for each subject were created by an experienced radiologist (G.C. with five years of experience) and checked by a senior neuroradiologist (Y.D. with 11 years of experience). 3D T1w images were nonlinearly registered to T2w or FLAIR images on which lesion mask were drawn for every subject, and the backward transformation matrix was obtained. Then T1w image was segmented using SPM12 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK) and the forward transformation matrix from T1w space to Montreal neurological institute (MNI) space was obtained. Finally lesion masks were normalized to

MNI space using the above backward and forward transformation matrices. The lesion probability map was created in the MNI space and lesion volumes were calculated in native space in the study.

Hippocampus volume, diffusion and functional measures

3D T1 images of 721 subjects were successfully processed using Freesurfer 6.0 software (http://surfer.nmr.mgh.harvard.edu/). The “recon-all” pipeline was used for preprocessing, including (1) Talairach-transformation, (2) intensity normalization, (3) skull stripping, (4) gray matter, white matter (WM) and CSF segmentation, (5) extraction of hippocampus volume using a non-linear warping atlas. Additionally, a visually checking of Talairach-transformation and manually correction (including 6 MS, 8 NMOSD and 9 HC) was conducted to ensure segmentation accuracy.

Diffusion data were processed using FMRIB’s Diffusion Toolbox (FDT, FSL 6.0.1, www.fmrib.ox.ac.uk/fsl). Head-motion and eddy currents were corrected, using the FMRIB's linear registration tool (FLIRT) to align all images to the first b0 image[16]. FA and MD images were registered to T1-weighted images after brain-extraction using an affine alignment[17]. FA and MD values were calculated from each voxel’s tensor, and averaged across regions as defined in the Automated Anatomical Labeling (AAL) atlas[18].

Resting-state fMRI data were processed using SPM12 and Data Processing and Analysis for Brain Imaging (DPABI, Version 4.4) software[19]. Amplitude of Low Frequency Fluctuation (ALFF) was used to measure

regional spontaneous activity, which was defined as the total power in the 0.01-0.1Hz frequency band. Voxel-based functional connectivity matrix was constructed using Pearson correlation and binarized with a threshold of 0.25. Degree centrality (DC) was calculated using the binarized functional network. The ALFF and DC values were transformed into Z-scores for statistical analysis.

Hippocampal subfield volume measurement

The hippocampus was automatically segmented into 13 subfields in each hemisphere (Figure 2), based on a tetrahedral mesh probabilistic atlas and a Bayesian algorithm[20] in Freesurfer 6.0 software. The alveus was discarded in this study due to small size and unreliable segmentation. Cornu ammonis 2 (CA2) and CA3 fields were combined because of unclear borders contrast [20]. Further details about the segmentation method are provided in Iglesias et al[20].

Validation

To exclude the disease phase influence, patients in the non-relapsing phase were treated as a subgroup. To exclude multi-center effects and improve data heterogeneity, the single largest center (Beijing Xuanwu hospital) data was selected to validate the results. Additionally, only patients without hippocampal lesions were included for analysis to exclude an effect of hippocampal lesions on MRI measurements. To exclude its influence between MS and NMOSD, disease duration was controlled as a covariate.

Statistical analysis

Statistical analyses were performed using SPSS software (version 22.0) and Matlab (2013a, MathWorks). The center effect, age and sex were firstly controlled by mixed linear regression modelling for MRI measures, and total intracranial volume was also considered for hippocampal volume. The corrected measure was calculated as residual plus mean of the corresponding measure, which was used for subsequent analysis.

Sex difference between groups was analyzed using a chi-square test. Group differences in MRI measures of the hippocampus between three groups were examined by analysis of variance (ANOVA). Spearman correlation was performed for correlation analysis, with 95% confidence interval (CI)[21]. False discovery rate (FDR) correction was performed for multiple comparisons (including the ANOVA post-hoc comparisons and comparisons across multiple MRI measures). Logistic regression was conducted to explore the discrimination ability of each MRI measure for MS and HC, NMOSD and HC, MS and NMOSD. The area under the receiver operator characteristics curve (AUC) were obtained to evaluate the model performance using a 10-fold nested cross-validation with 100 outer loop iterations to obtain the average value and standard deviation (refer to https://github.com/ZFL15/ROC1.git). The significant level was set at 0.05.

Results

Demographics and clinical information

As shown in Table 1, NMOSD patients were older, showed a higher female/male ratio, and higher EDSS scores and longer disease duration than MS patients. Of the 163 NMOSD patients tested, 102 (62.6%) were APQ4-IgG positive. Significantly lower CVLT and PASAT scores were observed in both MS and NMOSD groups compared with HC. BVMT scores lower in NMOSD compared to HC and MS.

Hippocampal lesion

In total, 236 MS and 130 NMOSD patients had brain lesions. The average brain lesion volume in MS was significantly higher than NMOSD (MS: 14.2±25.8 ml, NMOSD: 4.5±7.5ml, $P<0.001$). Twelve MS patients had hippocampal lesions, while no NMOSD patients had hippocampal lesions. The lesion probability map shows higher lesion probability in MS than NMOSD (Figure e-1).

Hippocampal volume, diffusion and functional measures

As shown in Table e-4 and Figure 3, lower volume, FA and greater MD of bilateral hippocampus were observed in both MS and NMOSD compared to HC and in MS compared to NMOSD ($P<0.001$). No group differences were observed for ALFF or DC of the hippocampus ($P>0.05$).

Hippocampal subfield volume

As shown in Figure 2 and Table e-4, MS patients showed significantly lower

subfield volumes including bilateral tail, subiculum, CA1, presubiculum, molecular layer, granule cell layer of the dentate gyrus (GC-DG), CA3, CA4, fimbria, hippocampal amygdala transition area (HATA), and right parasubiculum, yet greater volume in the fissure compared to HC. NMOSD demonstrated significantly lower hippocampal subfields volumes including bilateral tail, CA1, molecular layer, GC-DG, CA3, CA4 and HATA, yet greater volume of in bilateral fissure volume compared to HC. Comparing to NMOSD, MS showed significantly lower volumes of the bilateral tail, subiculum, presubiculum, molecular layer, GC-DG, fimbria, left HATA, and right CA1.

Correlation between MRI measurements and lesion volumes

In MS, lesion volume was correlated with the volume, FA, MD of the bilateral hippocampus, DC in the left hippocampus, ALFF in the right hippocampus, as well as several subfields volumes (e.g., tail, subiculum, CA1; R [correlation coefficient] =-0.641~0.595, all $P<0.05$). In NMOSD, lesion volume was correlated with FA of the bilateral hippocampus, the volume and MD of right hippocampus, right CA1 and molecular layer (R=-0.275~0.261, all $P<0.05$). Details see Figure 4 and Table e-5.

Correlation between MRI measurements and clinical status

In MS, disease duration was significantly correlated with the bilateral hippocampal volume, FA, MD, left hippocampus ALFF, and the volume of several hippocampal subfields (e.g., tail, molecular layer and fimbria; R=-0.402~0.328, all $P<0.05$). EDSS was correlated with bilateral volume, FA,

MD, and ALFF in the hippocampus, and the volume of several hippocampal subfields (e.g., molecular layer, GC-DG, fimbria; R=-0.286~0.314, all P<0.05). In NMOSD, disease duration was correlated with FA of the hippocampus (Left: R=-0.226, 95% CI [-0.368,-0.080], P=0.034, Right: R=-0.234, 95% CI [-0.373,-0.079], P=0.034). No association between EDSS and MRI measures was observed in NMOSD (P>0.05). Details see Figure 4 and Table e-6.

Correlation between MRI measurements and cognition scores

In MS, significant correlations were only observed between PASAT scores and the volume, FA, MD of the whole hippocampus, and several hippocampal subfield volumes in MS (e.g., subiculum, CA1, molecular layer; R=-0.444~0.498, all P<0.05). No significant correlation was observed in NMOSD. Details see Figure 4 and Table e-7.

Group discrimination

As shown in Table e-8, the hippocampal measures showed poor discriminative power for MS and NMOSD (AUC<0.6). FA and MD showed fair discriminative power for MS and HC (AUC>0.8),, and for NMOSD and HC (AUC>0.7).

Validation

First, the results in the non-relapsing phase of both patient subgroups were largely consistent with the whole group analyses (Table e-9). Second, separate subgroup analysis in the single largest center was very similar as compared to the whole group analysis (Table e-10). Third, only patients

without hippocampal lesions were entered for analysis. The results were largely consistent with the whole group analysis (Table e-11). Finally, the results of partial correlation with disease duration as covariate were largely consistent with primary results (see Table e-12~e-15).

Discussion

In the current study, we demonstrated differential patterns of hippocampal structural and diffusional alterations in MS and NMOSD, without significant functional MRI alterations in either disease. Hippocampal diffusion measures and subfield volume correlated with clinical disability and cognitive impairment in both MS and NMOSD, and diffusion measures can better distinguish MS from NMOSD.

Compared to HC, MS showed overall hippocampal atrophy, which is consistent with previous studies[22, 23]. The lower hippocampal volume reflects neuropathological processes, including lower dendritic density, neuronal loss, and demyelination in MS[10]. Hippocampal atrophy was also observed in NMOSD in the current study, though to a milder degree as compared to MS, which was consistent with the previous study[24]. However, previous studies reported controversial results [25], which may due to the different recruitment criteria (e.g., ethnicity, disease status) and segmentation methods.

Diffusion alterations of the hippocampus were observed in both MS and NMOSD, implying hippocampal demyelination and axonal damage as common features of two diseases[6, 24]. MS showed more severe hippocampal microstructural abnormalities than NMOSD, and these differences might help discriminate MS from NMOSD. Our study showed no change in ALFF (functional activity) or DC (functional connectivity) of the hippocampus in either MS or NMOSD. However, previous study of small sample size reported

increased synchronization in hippocampus in NMOSD[26], which may due to the heterogeneity of the patient groups. Functional impairment and adaptation may coexist in this cohort which may have cancelled out each other.

We also identified different regional atrophy patterns within the hippocampus between MS and NMOSD. The subiculum, presubiculum, and fimbria showed significant differences in MS compared to HC, while no significant atrophy was observed in NMOSD, highlighting the importance of these subfields in differentiating MS-related versus NMOSD-related damage. The complex of subiculum and presubiculum is important for hippocampal circuitry (memory, motivation, reward and stress response), which is one of the most severely damaged subfields in MS[27, 28]. As a WM structure, the fimbria extends from the alveus and eventually forms the fornix. Fimbria-fornix connects the left and right hippocampus, and has been associated with memory ability[29]. The lower volume of the fimbria in MS, rather than NMOSD, indicates that the WM damage was more severe in MS.

Total WM lesion volume was significantly associated with hippocampal volume in MS, slightly in NMOSD; although associated with diffusion abnormalities in both diseases. These findings suggest brain lesion is a driving factor for hippocampus atrophy (neurodegeneration) in MS, but not in NMOSD, but contribute to demyelination or axonal injury in both MS and NMOSD.

The total hippocampus volume, FA and MD were correlated with disease

duration in MS, while only FA correlated with disease duration in NMOSD. These results indicated that microstructural alterations of the hippocampus reflect disease progression in both diseases, while hippocampal atrophy is more relevant in MS. Hippocampus measures were also correlated with EDSS in MS but not in NMOSD, implying that the atrophy and microstructural alterations of hippocampus are sensitive imaging markers for clinical disability in MS. Hippocampus subfield volume analyses confirmed the total hippocampus volume findings, with subfield volumes generally correlating with disease duration and EDSS in MS. However, Galego et al found no relation between hippocampal volume and EDSS or disease duration in primary progressive MS patients (PPMS)[30], which is inconsistent with our findings. One possible explanation is that disability in PPMS was more associated with spinal cord injury[31].

Hippocampal diffusion parameters that correlate with cognition was reported in MS[6], which is consistent with our findings showing the correlation between the auditory processing speed ability and hippocampal diffusion alterations. Additionally, the total volume of the hippocampus and several subfield volumes significantly correlated to PASAT scores in our study. Bozzali et al reported that the anatomical connectivity mapping in the hippocampus was associated with PASAT scores in MS[32]. Taken together, these findings suggest that hippocampal diffusion and structural alterations might be important markers for evaluating auditory processing speed ability in

MS. Additionally, several findings which not survived the FDR correction might be still clinical relevance, such as the correlations between hippocampal subfield volumes (CA1, presubiculum, tail, presubiculum, fimbria, subiculum) and cognitive scores. Previous studies reported that the atrophy of the subiculum correlated with visuospatial memory, verbal memory, and memory acquisition in MS[11, 27], which was different from our results. The discordance may due to different segmentation methods and patient selection. Several previous studies reported association between the volume of hippocampal subfields and cognitions, such as CA1 with spatial memory[33], and presubiculum with verbal learning memory[34]. These different patterns imply slightly different substrates of cognitive impairment and the importance of hippocampal subfields for different domains of cognitive impairment[31], although no such correlation was observed in our study.

There are several limitations in this study. First, this is a retrospective study with limited cognitive assessment (such as lack of Symbol Digit Modalities Test scores) and other phenotypes of MS patients (PPMS, Secondary progressive MS), precluding a more comprehensive assessment of hippocampal function concerning subfield damage. Further prospective studies with comprehensive information are warranted to investigate the clinical importance of hippocampal-imaging markers. Second, the MRI parameters and subjects were heterogeneous across centers (see Table e-1, Table e-3, and Figure e-2), which would cause scanner/MRI protocol bias. However, our validation results

showed consistent findings, suggesting that our findings are robust and can be generalized. And hippocampal lesion frequency may have been underestimated. Third, due to the small size of the hippocampus, we could not study diffusion and functional alterations within hippocampal subfields, which would need higher resolution MRI and more sophisticated analysis methods to define subfield structure and function. In addition, 1mm resolution might not be optimal for automatic segmentation of hippocampal subfields, and the results of hippocampal subfields should be interpreted with caution [20,35], even though the Bayesian segmentation algorithm warps anatomic labels with a 0.13 mm resolution with subvoxel accuracy. Further studies with submillimeter resolution are warrant to validate the current findings. Last, we just selected ALFF and DC to account for hippocampus functional alterations. Various metrics (e.g., node degree, betweenness centrality) could characterize the hippocampus at regional and global levels, which would be examined in the future.

Conclusion

Differential patterns of morphometric and diffusional hippocampal abnormities were identified in MS and NMOSD. Hippocampal morphometric features, particularly hippocampal subfield volumes, and diffusion measures can serve as potential objective imaging biomarkers to monitor disease progression and cognitive impairment in these two diseases.

Author contributions

Fenglian Zheng and Zhizheng Zhuo analyzed the data. Fenglian Zheng and Yaou Liu designed the study. Yunyun Duan and Guanmei Cao created the lesion mask. Sven Hallar, Frederik Barkhof and Yaou Liu reviewed and edited the manuscript. Other authors contributed to patient recruitment and data analysis.

Acknowledgments

We acknowledged all the staff and patients in the participating centers for their contributions to this study. Professor Frederik Barkhof is supported by the NIHR biomedical research center at UCLH.

Data availability statement

The data are available to qualified researchers from the corresponding author upon written request.

Study funding

This work was supported by the National Science Foundation of China (Nos. 81870958 and 81571631), the Beijing Natural Science fund (No.7133244), the Beijing Nova Program (xx2013045) and the Shanghai new star of Medical Court (2018, H. L.). F.B. is supported by the NIHR biomedical research center at UCLH.

Declaration of conflicting interests

Fenglian Zheng, Yuxin Li, Zhizheng Zhuo, Yunyun Duan, Guanmei Cao, Xiaolu Xu, Decai Tian, Xinghu Zhang, Kuncheng Li, Fuqing Zhou, Muhua Huang, Haiqing Li, Yongmei Li, Chun Zeng, Ningnannan Zhang, Jie Sun,

Chunshui Yu, Xuemei Han, Sven Hallar, and Yaou Liu declare that there is no conflict of interest.

Professor Frederik Barkhof is an advisor to Bayer-Schering, Biogen-Idec, GeNeuro, Ixico, Merck-Serono, Novartis and Roche. He has received grants or grants pending from the Amyloid Imaging to Prevent Alzheimer's Disease (AMYPAD) initiative, the Biomedical Research Centre at University College London Hospitals, the Dutch MS Society, ECTRIMS–MAGNIMS, EU-H2020, the Dutch Research Council (NWO), the UK MS Society, and the National Institute for Health Research, University College London. He has received the remuneration for development of educational presentations from Ixico and for his institution from Biogen-Idec and Merck. He is a member of the editorial board of Radiology, Brain, European Radiology, Multiple Sclerosis Journal and Neurology.

References

1. Noseworthy JH, Lucchinetti C, Rodriguez M and Weinshenker BG. Multiple sclerosis. *N Engl J Med*. 2000; 343: 938-952. DOI:10.1056/NEJM200009283431307.

2. Höftberger R and Lassmann H. Inflammatory demyelinating diseases of the central nervous system. *Handb Clin Neurol*. 1991; 145: 263-283. DOI:10.1016/B978-0-12-802395-2.00019-5.

3. Toth E, Farago P, Kiraly A, et al. The Contribution of Various MRI Parameters to Clinical and Cognitive Disability in Multiple Sclerosis. *Front Neurol*. 2018; 9: 1172. DOI: 10.3389/fneur.2018.01172.

4. Vanotti S, Cores EV, Eizaguirre B, Melamud L, Rey R and Villa A. Cognitive performance of neuromyelitis optica patients: comparison with multiple sclerosis. *Arq Neuro-Psiquiat*. 2013; 71: 357-361. DOI: 10.1590/0004-282x20130038.

5. Squire LR, Stark CE and Clark RE. The medial temporal lobe. *Annual Review of Neuroscience*. 2004; 27: 279-306. DOI:10.1146/annurev.neuro.27.070203.144130.

6. Planche V, Ruet A, Coupé P, et al. Hippocampal microstructural damage correlates with memory impairment in clinically isolated syndrome suggestive of multiple sclerosis. *Mult Scler*. 2016; 23: 1214-1224. DOI: 10.1177/1352458516675750.

7. Kawahara Y, Ikeda M, Deguchi K, et al. Cognitive and Affective Assessments of Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO) Patients Utilizing Computerized Touch Panel-type Screening Tests. *Intern Med*. 2014; 53: 2281-2290. DOI:10.2169/internalmedicine.53.2571.

8. Planche V, Koubiyr I, Romero JE, et al. Regional hippocampal vulnerability in early

multiple sclerosis: Dynamic pathological spreading from dentate gyrus to CA1. *Hum Brain Mapp*. 2018; 39:1814-1824. DOI:10.1002/hbm.23970

9. Cacciaguerra L, Pagani E, Mesaros S, et al. Dynamic volumetric changes of hippocampal subfields in clinically isolated syndrome patients: A 2-year MRI study. *Mult Scler*. 2018; 25: 1232-1242. DOI:10.1177/1352458518787347

10. Rocca MA, Longoni G, Pagani E, et al. In vivo evidence of hippocampal dentate gyrus expansion in multiple sclerosis. *Hum Brain Mapp*. 2015; 36: 4702-4713. DOI:10.1002/hbm.22946

11. Sicotte NL, Kern KC, Giesser BS, et al. Regional hippocampal atrophy in multiple sclerosis. *Brain*. 2008; 131: 1134-1141. DOI:10.1093/brain/awn030

12. Finke C, Heine J, Pache F, et al. Normal volumes and microstructural integrity of deep gray matter structures in AQP4+ NMOSD. *Neurol Neuroimmunol Neuroinflamm*. 3: e229. DOI:10.1212/NXI.0000000000000229.

13. Delis D. California verbal learning test. *Adult version Manual Psychological Corporation*. 2000.

14. Benedict R. Brief visuospatial memory test-revised: professional manual. Odessa, Florida: Psychological Assessment Resources. Inc, 1997.

15. Gronwall DM. Paced auditory serial-addition task: a measure of recovery from concussion. *Percept Mot Skills*. 1977; 44: 367-373. DOI: 10.2466/pms.1977.44.2.367.

16. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*. 2004; 23:208-219. DOI:10.1016/j.neuroimage.2004.07.051

17. Jenkinson M and Smith S. A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*. 2001; 5: 143-156. DOI:10.1016/s1361-8415(01)00036-6

18. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002; 15: 273-289. DOI: 10.1006/nimg.2001.0978.

19. Yan C and Zang Y. DPARSF: A MATLAB Toolbox for "Pipeline" Data Analysis of Resting-State fMRI. *Frontiers in Systems Neuroscience*. 2010; 4: 13. DOI:10.3389/fnsys.2010.00013.

20. Iglesias JE, Augustinack JC, Nguyen K, et al. A computational atlas of the hippocampal formation using ex vivo , ultra-high resolution MRI: Application to adaptive segmentation of in vivo MRI. *Neuroimage*. 2015; 115: 117-137. DOI:10.1016/j.neuroimage.2015.04.042

21.Harrington D, D'Agostino RB Sr, Gatsonis C, et al. New Guidelines for Statistical Reporting in the Journal. N Engl J Med. 2019; 381:285-286. DOI: 10.1056/NEJMe1906559.

22. Hamilton AM, Forkert ND, Yang R, et al. Central nervous system targeted autoimmunity causes regional atrophy: a 9.4T MRI study of the EAE mouse model of Multiple Sclerosis. *Sci Rep*. 2019; 9: 8488. DOI: 10.1038/s41598-019-44682-6.

23. Anderson VM, Fisniku LK, Khaleeli Z, et al. Hippocampal atrophy in relapsing-remitting and primary progressive MS: a comparative study. *Mult Scler*. 2010;

16: 1083-1090. DOI: 10.1177/1352458510374893.

24. Chen X, Fu J, Luo Q, et al. Altered volume and microstructural integrity of hippocampus in NMOSD. *Mult Scler Relat Disord*. 2019; 28: 132-137. DOI: 10.1016/j.msard.2018.12.009.

25. Hyun JW, Park G, Kwak K, et al. Deep gray matter atrophy in neuromyelitis optica spectrum disorder and multiple sclerosis. *European Journal of Neurology*. 2017; 24: 437-445. DOI:10.1111/ene.13224

26. Rueda-Lopes FC, Pessôa FMC, Tukamoto G, et al. Default-Mode Network And Deep Gray-Matter Analysis In Neuromyelitis Optica Patients. *Journal of Neuroradiology*. 2018; 45:256-260. DOI: 10.1016/j.neurad.2018.01.053.

27. Longoni G, Rocca MA, Pagani E, et al. Deficits in memory and visuospatial learning correlate with regional hippocampal atrophy in MS. *Brain Struct Funct*. 2015; 220: 435-444. DOI: 10.1007/s00429-013-0665-9.

28. Herman JP and Mueller NK. Role of the ventral subiculum in stress integration. *Behavioural Brain Research*. 2006; 174: 215-224. DOI:10.1016/j.bbr.2006.05.035

29. Hao S, Tang B, Wu Z, et al. Forniceal deep brain stimulation rescues hippocampal memory in Rett syndrome mice. *Nature*. 2015; 526: 430-434. DOI: 10.1038/nature15694.

30. Galego O, Gouveia A, Batista S, Moura C and Machado E. Brain atrophy and physical disability in primary progressive multiple sclerosis: A volumetric study. *Neuroradiology Journal*. 2015; 28: 354. DOI:10.1177/1971400915594984

31. Lukas C, Knol DL, Sombekke MH, et al. Cervical spinal cord volume loss is related

to clinical disability progression in multiple sclerosis. *Journal of Neurology Neurosurgery & Psychiatry*. 2015; 86: 410-418. DOI:10.1136/jnnp-2014-308021

32. Bozzali M, Spano B, Parker GJ, et al. Anatomical brain connectivity can assess cognitive dysfunction in multiple sclerosis. *Mult Scler*. 2013; 19: 1161-1168. DOI: 10.1177/1352458512474088.

33. Tsien JZ, Huerta PT and Tonegawa S. The Essential Role of Hippocampal CA1 NMDA Receptor–Dependent Synaptic Plasticity in Spatial Memory. *Cell*. 1996; 87: 1327-1338. DOI: 10.1016/s0092-8674(00)81827-9.

34. Garcíagarcía R, CruzGómez ÁJ, Urios A, et al. Learning and Memory Impairments in Patients with Minimal Hepatic Encephalopathy are Associated with Structural and Functional Connectivity Alterations in Hippocampus. *Scientific Reports*. 2018; 8: 9664. DOI:10.1038/s41598-018-27978-x.

35. Wisse LEM, Chetelat G, Daugherty AM, et al. Hippocampal subfield volumetry from structural isotropic 1 mm^3 MRI scans: A note of caution. *Hum Brain Mapp*. 2020;42:539-550. DOI:10.1002/hbm.25234