Progressive Volume Atrophy in Hippocampal Subfields and the Correlation with Cognition in Alzheimer's Disease and Mild Cognitive Impairment

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Progressive volume atrophy in hippocampal subfields and the correlation with cognition in Alzheimer's Disease and Mild Cognitive Impairment

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Abstract

Objective: To assess the progression of volume changes in hippocampus and its subfields of patients with Alzheimer's disease (AD) and mild cognitive impairment (MCI), and to explore the association of the hippocampus and its subfields volumes with cognitive function.

Methods: Five groups of participants including 35 normal controls (NC) persons, 30 MCI patients, 30 Mild AD patients, 30 Moderate AD patients and 8 Severe AD patients received structural MRI brain scans. Freesurfer6.0 was used for automatically segmentation of MRI, and the left and right hippocampus were respectively divided into 12 subfields. By statistical analysis, the volumes of hippocampus and its subfields were compared between the five groups, and the correlation of the volumes with Mini-mental State Examination (MMSE) score was analyzed.

Result & Conclusion: In the disease, each hippocampal subfield shows an uneven atrophy trajectory; The volumes of the subiculum and presubiculum are significantly different between Mild AD and MCI, which can contribute to the early diagnosis of AD; Parasubiculum is the least sensitive subfield for volume atrophy of AD, while subiculum, presubiculum, CA1, molecular_layer_HP and fimbria show much more significant volume changes. Meanwhile the volumes of these five subfields are positively correlated with MMSE, which may help in stage division of AD; Compared with the right hippocampus, the volume atrophy on the left side is more significantly, and the volumes are more significantly correlated with MMSE, So the left hippocampus and its subfields may provide a higher reference value for the clinical evaluation of AD than the right side.

Keywords: Alzheimer's disease, Mild cognitive impairment, Hippocampal subfields, MRI, Atrophy

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is a neurodegenerative disease characterized by progressive cognitive dysfunction and behavioral impairment [1]. It is characterized by two neuropathological hallmarks, the accumulation of amyloid-β(Aβ) deposits and neurofibrillary tau tangles [2], which ultimately lead to neuronal atrophy and cognitive decline. Mild cognitive impairment (MCI) is a state of cognitive impairment between normal people and AD, which is considered as the prodromal stage of AD [3]. With the development of neuroimaging, abnormal intracranial gray matter volume has been confirmed in AD and MCI patients [4-6], especially atrophy of hippocampal volume [7-9]. So the hippocampus is considered to be a relatively reliable imaging marker for the progression of AD disease, and hippocampal volume has been an effective method for the analysis of AD pathology. But it has been found in the relevant clinical studies that the volume change of the hippocampus is related to many other neurological diseases, such as temporal lobe epilepsy and depression, which can also lead to the atrophy of hippocampus [10-11]. Therefore, the study of hippocampal subfields is very important for the diagnosis and differentiation of diseases related to hippocampal atrophy. It is acknowledged that hippocampus is heterogeneous and can be divided into some subfields with different functions, connectivity to other brain regions and vulnerability to disease [12-14]. However, most current studies have analyzed the volume of the whole hippocampus [15-17] or the volume of the hippocampal head, body and tail [18-19], and the hippocampus was not finely divided into multiple subregions. In addition, these studies usually only used the data of AD, MCI and healthy control group for comparison and analysis [20-22], without detailed grouping of AD patients by disease severity, and no horizontal analysis of the different trajectories of different hippocampal subfields volumes in the entire course of AD.

In this study, all subjects were divided into 5 groups, namely the MCI, Mild AD, Moderate AD, Severe AD and NC (Normal Control) group. The left and right sides of the hippocampus are both divided into 12 subfields. And the periodic changes of hippocampus and hippocampal subfields

volume in AD and MCI patients were evaluated comprehensively by MRI. In addition, we explore the correlation of hippocampus and hippocampal subfields volume with cognitive function at each stage of the disease.

2. Material and Methods

2.1. Participants

A total of 138 subjects were enrolled, including 35 in the NC group, 35 in the MCI group, 30 in the Mild AD group, 30 in the Moderate AD group, and 8 in the Severe AD group. The subjects for the NC, MCI, Mild AD and Moderate AD groups were derived from the 1.5 Tesla datasets of ADNI-1, 2, and 3 in the ADNI (Alzheimer's Disease Neuroimaging Initiative) database of the Alzheimer's Neuroimaging Program. The ADNI (http://adni.loni.usc.edu) is a longitudinal multicenter study designed to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. Since its launch more than a decade ago, the landmark public-private partnership has made major contributions to AD research, enabling the sharing of data between researchers around the world. A detailed description of the inclusion criteria for this study can be found in the ADNI protocol. We selected 35 normal controls in the ADNI database as the NC group with a MMSE (Mini-Mental State Examination) [23] score of 24 to 30 and a CDR (Clinical Dementia Rating) [24] of 0. And 35 patients with MCI who had an MMSE score of 24 to 30 and a CDR of 0.5 were selected as the MCI group. What's more, patients in AD group in ADNI database were further grouped according to MMSE and CDR. Among them, 30 AD patients with MMSE score of 21-26 and CDR score of 1 were selected as Mild AD group, and 30 AD patients with MMSE score of 10-20 and CDR score of 2 were selected as Moderate AD group.

The subjects of Severe AD group were collected from 8 patients with AD in the neurosurgery department of Chinese PLA General Hospital from June 2015 to September 2018. All of whom met the diagnostic criteria of possible AD published by the NINCDS-ADRDA [25]; the MMSE score was 0 ~ 9 and CDR score was 3; Other neuropsychiatric disorders other than AD are excluded.

2.2. Neuropsychological test

All subjects underwent MMSE and CDR examinations, which are two neuropsychologic scales commonly used for AD assessment and screening.

2.3. MRI acquisition

For each subject in NC, MCI, Mild AD and Moderate AD groups screened in the ADNI database, a scanner from Philips medical system was used to collect T1 images of 1.5T by sagittal 3D MP-rage sequence. And the detailed description of the ADNI data acquisition agreement.

For each subject in the Severe AD group, T1-images of 1.5T were collected by the Siemens Medical System in the radiology department of Chinese PLA General Hospital. Field of view (FOV) : 256×256mm; Echo time (TE) : 3.08ms; Repeat time (TR) : 2000ms; Layer thickness: 1mm, layer spacing: 0mm; Turning Angle (FA) : 8.0 degrees. The scanning azimuth is parallel to the forward and backward alignment.

2.4. Hippocampal subfields segmentation and volume estimation

The structural MRI was processed by FreeSurfer 6.0 (http://freesurfer.net).

Firstly, the hippocampus was separated from the brain tissue. Head motion correction was performed on subjects' T1 images, and a Hybrid Watershed/Surface deformation algorithm was used to remove the non-brain tissue to achieve the skull stripping. After that, the resulting image is further registered into Talairach standard space. And the volumes of the left and right hippocampus and the eTIV (estimated Total Intracranial Volume) of each subject were obtained by automatic calculation.

The hippocampus was then divided into many subfields. The Bayesian statistical model with Markov Random Field priori was used to estimate the division of each subfield, and by maximizing the posterior probability of segmentation, the left and right hippocampus were automatically divided into 12 subfields: hippocampal_tail, subiculum, CA1, hippocampal-fissure, presubiculum, parasubiculum, molecular_layer_HP, GC-ML-DG, CA2-3, CA4, fimbria and HATA [26]. The volume of each hippocampal subfield was automatically quantitatively evaluated by FreeSurfer6.0. The accuracy of this method has been verified [27], and the segmentation results are shown in figure 1.

In order to eliminate the influence of individual eTIV in the analysis, the volume of hippocampus and hippocampal subfields have been standardized, i.e. standardized volume = (individual actual volume / individual eTIV) × average eTIV.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 19.0. The count data (e.g. gender) is represented by the number of cases, and the Chi-square test is used for comparison between groups. The measurement data is tested by Kolmogorov-Smirnov for normality. If the sample does not conform to the normal distribution, the appropriate conversion method is used to improve the data distribution. Or if the sample conforms to the normal distribution, it is expressed as mean ± standard deviation ($\bar{x} \pm s$). And then the Levene's was used for the homogeneity test of variance. The comparison between groups was performed by ANOVA one-way analysis of variance. Finally, the results of multiple comparisons were corrected by LSD post-test. In addition, the correlation analysis of the hippocampus and hippocampal subfields volume with MMSE scores was performed by Pearson correlation analysis, and the correlation analysis

with CDR was performed using Kendall correlation analysis. $P < 0.05$ indicates statistical difference, and $P < 0.01$ indicates significant statistical difference.

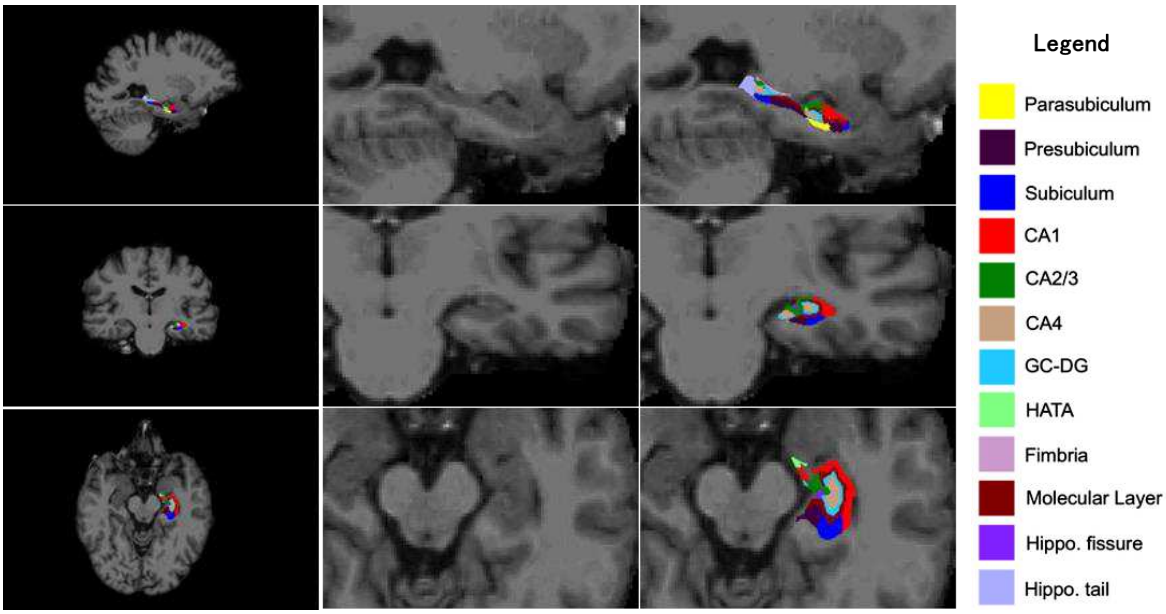


Figure 1 Segmentation of hippocampal subfields by Freesurfer 6.0

3. Results

3.1. Demographic and neuropsychological data

As can be seen in Table 1, the gender distribution among NC, MCI, Mild AD, Moderate AD and Severe AD groups (chi-squared =1.569, P=0.814) was not statistically significant. There was no statistical difference in age distribution between the four groups except Severe AD group (F=0.834, P=0.478), while there was significant statistical difference in age distribution between Severe AD group and the other groups (P<0.01), so age was taken as a covariable in the follow-up analysis. There were significant differences in MMSE and CDR scores between the five groups (P<0.01), and there were significant differences between any two groups (P<0.01).

3.2. Comparison of hippocampus and hippocampal subfields volume

3.2.1. Comparison of the whole hippocampal volume

The statistical comparison of the left and right whole hippocampal volumes is shown in Table 2 and Figure 2.

Comparison between the all five groups: The bilateral whole hippocampal volumes both showed significant statistical difference among the five groups (left side: F=11.172, P<0.01; Right side: F=8.914, P<0.01).

Comparison with NC group: The volumes of the bilateral whole hippocampus in MCI group were both found decreased compared with NC group (P<0.05). And the bilateral whole hippocampal volumes of AD group were both significantly reduced (P<0.01).

Comparison with the MCI group: There was no statistically significant difference in the bilateral whole hippocampal volumes between Mild AD group and MCI group (P>0.05). Compared with MCI group, the bilateral hippocampal volumes of Moderate AD and Severe AD group was significantly reduced (P<0.01).

Comparison between the groups of Mild AD, Moderate AD and Severe AD: There was no statistically significant difference in the bilateral whole hippocampal volumes between the groups of Mild AD, Moderate AD and Severe AD (P>0.05).

Table 1 Demographic and neuropsychological data of the five groups of participants

	NC (n=35)	MCI (n=35)	Mild AD (n=30)	Moderate AD (n=30)	Severe AD (n=8)	P
Male/Female	18/17	17/18	13/17	15/15	5/3	0.900
Age	76.3 ± 5.3	74.7 ± 6.8	73.9 ± 7.9	76.7 ± 8.3	59.9 ± 4.5	0.000**
MMSE	28.71 ± 1.05	26.84 ± 1.98	23.67 ± 1.81	14.97 ± 3.18	1.50 ± 2.51	0.000**
CDR	0	0.5 ± 0	1.0 ± 0	2.0 ± 0	3.0 ± 0	0.000**

Table 2 Comparison of hippocampus and hippocampal subfields volume between the five groups of participants

	NC (n=35)	MCI (n=35)	Mild AD (n=30)	Moderate AD (n=30)	Severe AD (n=8)	F	P
lh_Whole_hippocampus	3206.95±612.42	2838.1±512.58 [a]	2532.96±658.00 [ab]	2326.44±673.96 [ab]	2061.88±579.81 [ab]	11.172	0.000**
rh_Whole_hippocampus	3312.29±612.2	2940.47±469.15 [a]	2632.84±699.04 [ab]	2499.20±842.17 [ab]	2207.07±499.52 [ab]	8.914	0.000**
lh_hippocampal_tail	501.35±111.39	470.17±72.77	419.50±91.48[a]	384.74±93.29 [ab]	343.31±95.05 [ab]	8.076	0.000**
rh_ hippocampal_tail	535.88±103.35	492.06±94.95	440.30±98.30 [a]	427.25±125.78 [ab]	358.73±78.35 [ab]	7.330	0.000**
lh_subiculum	413.55±87.06	356.45±75.56 [a]	313.76±84.57 [ab]	272.01±81.31 [abc]	227.91±65.71 [abc]	16.588	0.000**
rh_ subiculum	413.91±82.68	359.93±64.38 [a]	308.62±82.25 [ab]	289.93±109.28 [ab]	246.92±61.87 [ab]	12.779	0.000**
lh_CA1	592.24±109.56	526.17±111.55 [a]	495.31±138.57 [a]	456.33±144.99 [ab]	386.17±118.12 [abc]	7.194	0.000**
rh_ CA1	616.61±115.70	554.47±85.15 [a]	515.65±148.01 [a]	472.66±160.45 [ab]	430.73±122.65 [ab]	6.566	0.000**
lh_hippocampal-fissure	166.13±39.08	172.96±38.89	164.25±48.19	143.09±43.91 [ab]	119.98±37.04 [ab]	4.076	0.004**
rh_ hippocampal-fissure	182.19±47.18	184.80±41.30	171.99±43.38	150.40±55.40 [ab]	141.59±49.51 [ab]	3.337	0.012*
lh_presubiculum	295.54±60.90	260.93±50.47 [a]	223.79±58.05 [ab]	200.32±62.12 [ab]	178.80±39.96 [abc]	13.392	0.000**
rh_ presubiculum	279.92±57.58	244.18±42.92 [a]	209.22±58.43 [ab]	207.13±81.33 [ab]	199.53±56.80 [ab]	7.973	0.000**
lh_parasubiculum	58.45±19.36	57.14±12.40	50.06±18.49 [a]	48.10±21.45	45.71±19.33 [ab]	2.025	0.094
rh_ parasubiculum	57.79±17.36	53.79±12.07	47.01±14.70 [a]	48.94±26.79 [a]	42.19±16.83 [a]	2.196	0.073*
lh_molecular_layer_HP	527.89±99.08	458.29±91.42 [a]	408.09±114.29 [a]	373.48±116.34 [ab]	324.45±98.15 [abc]	12.006	0.000**
rh_ molecular_layer_HP	543.93±103.47	474.27±78.80 [a]	421.52±121.39 [a]	396.79±135.91 [ab]	354.48±87.08 [ab]	10.018	0.000**
lh_GC-ML-DG	273.21±54.35	238.71±48.05 [a]	211.39±61.11 [a]	200.33±61.61 [ab]	185.88±62.96 [ab]	9.375	0.000**
rh_ GC-ML-DG	290.81±58.29	255.67±44.9 [a]	234.33±66.76 [a]	220.31±71.30 [ab]	194.14±44.26 [ab]	8.112	0.000**
lh_CA2-3	197.17±44.67	170.85±34.31 [a]	151.13±46.77 [a]	145.21±49.20 [ab]	134.95±42.24 [ab]	8.160	0.000**
rh_ CA2-3	214.70±49.01	189.63±33.09 [a]	171.72±54.25 [a]	162.51±56.19 [ab]	136.77±37.03 [ab]	7.372	0.000**
lh_CA4	238.20±46.49	211.35±40.25 [a]	188.58±54.01 [a]	177.59±54.90 [ab]	163.10±56.73 [ab]	8.586	0.000**
rh_ CA4	254.82±50.93	226.51±39.53 [a]	207.42±57.80 [a]	194.60±62.36 [ab]	167.78±38.97 [ab]	8.023	0.000**
lh_fimbria	54.64±24.46	38.92±23.25 [a]	28.77±15.81 [a]	27.89±16.33 [ab]	26.76±8.81 [ab]	9.820	0.000**
rh_ fimbria	47.99±17.66	38.21±20.96 [a]	31.35±20.59 [a]	33.70±29.65 [a]	31.61±9.65 [a]	3.370	0.012**
lh_HATA	54.69±15.51	49.12±13.53	42.60±17.64 [a]	40.43±14.03 [ab]	35.51±14.07 [ab]	5.540	0.000**
rh_ HATA	55.93±13.06	51.74±10.28	45.69±16.33 [a]	45.39±20.95 [a]	38.63±14.06 [ab]	3.594	0.006**

'lh' represents the left hippocampus and hippocampal subfields, 'rh' represents the right hippocampus and hippocampal subfields; volume unit is mm^3.

* indicates statistical difference between the five groups, P<0.05.

** indicates significant statistical difference between the five groups, P<0.01.

[a] indicates statistical difference compared to NC group, P<0.05.

[b] indicates statistical difference compared to MCI group, P<0.05.

[c] indicates statistical difference compared to Mild AD group, P<0.05.

[d] indicates statistical difference compared to Moderate AD group, P<0.05.

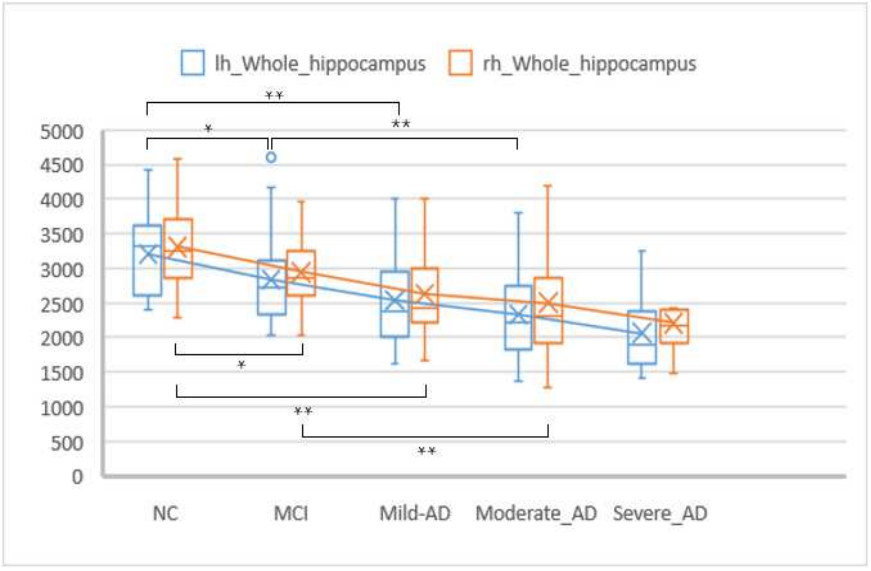


Figure 2 Comparison of the bilateral whole hippocampal volumes between five groups. *P<0.05, **P<0.01

Figure 3 Comparison of hippocampal subfields volumes between five groups. *P<0.05，**P<0.01

3.2.2. Comparison of hippocampal subfields volumes

Table 2 report the volumes of different hippocampal subfields of participants in each group and the results of relevant inter-group comparisons are showed in figure 3.

Comparison between the five groups: Except the right parasubiculum, all other hippocampal subfields showed significant differences in volume between the five groups (P<0.01).

Compared with NC group: The volumes of the bilateral hippocampal_tail, hippocampal-fissure, parasubiculum, HATA in MCI group were not significantly different from that in NC group (P>0.05). The volumes of bilateral CA1, presubiculum, GC-ML-DG, CA2-3, CA4 and fimbria were all found decreased (P<0.05), and the bilateral subiculum and molecular_layer_HP volumes decreased significantly (P<0.01). The volumes of bilateral hippocampal-fissure in Mild AD group were not significantly different from that in

NC group (P<0.05), and the volumes of the bilateral parasubiculum decreased (P<0.05), while the volumes of other hippocampal subfields decreased significantly (P<0.01). In addition, the volumes of bilateral parasubiculum and the left hippocampal-fissure in the Moderate AD and Severe AD groups reduced compared with the NC group, (P<0.05), and the volumes of all other hippocampal subfields was significantly reduced (P<0.01).

Comparison with the MCI group: Except for the reduced volumes of the bilateral subiculum and presubiculum (P<0.05), there was no statistical difference in the volume of the other hippocampal subfields in Mild AD group compared with the MCI group (P>0.05). There was no significant difference in the volume of the bilateral parasubiculum, right fimbria and right HATA in the Moderate AD group compared with the MCI group (P>0.05), but the remaining hippocampal subfields volumes were statistically different (P<0.05), in which the volumes of the bilateral subiculum, hippocampal-fissure, molecular_layer_HP and the left presubiculum, GC-ML-DG and CA4 decreased significantly (P<0.01). As for Severe AD group, the volumes of the parasubiculum and fimbria on the right side were significantly different (P<0.05) from those in the MCI group, and there were statistically significant decreases in the volumes of the bilateral hippocampal_tail, subiculum, CA1, molecular_layer_HP, and the left hippocampal-fissure, presubiculum, as well as the right CA2-3, CA4 (P<0.01).

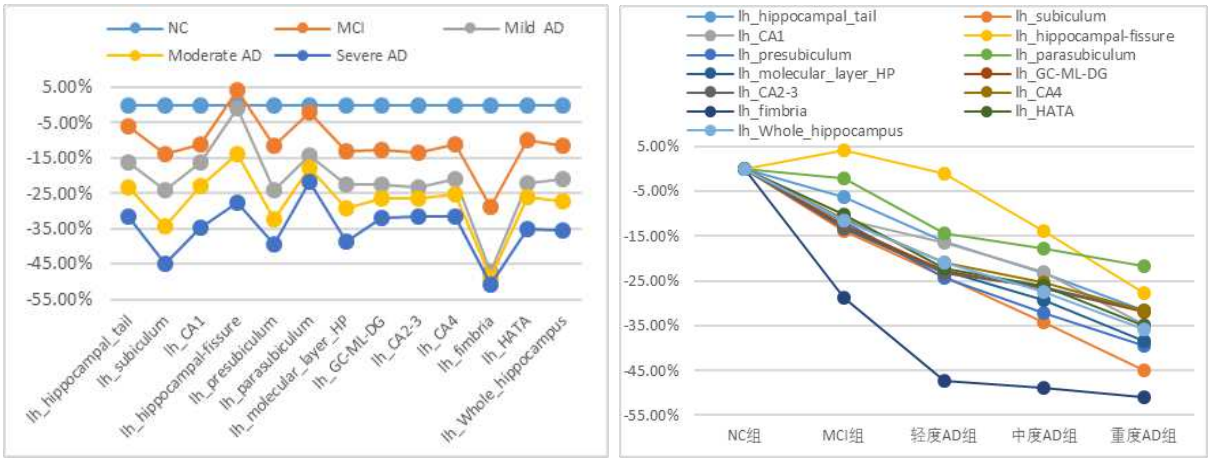
The comparison between Mild AD, Moderate AD, and Severe AD groups: Only the volume of the left subiculum in Moderate AD group decreased compared with that in Mild AD group (P<0.05). In the Severe AD group, the subiculum, presubiculum, CA1 and molecular_layer_HP on the left side all decreased compared with Mild AD group (P<0.05). And there was no statistically significant difference in all of the hippocampal subfields between Severe AD and Moderate AD groups (P>0.05).

3.3. Comparison of volume atrophy rates in hippocampal subfields

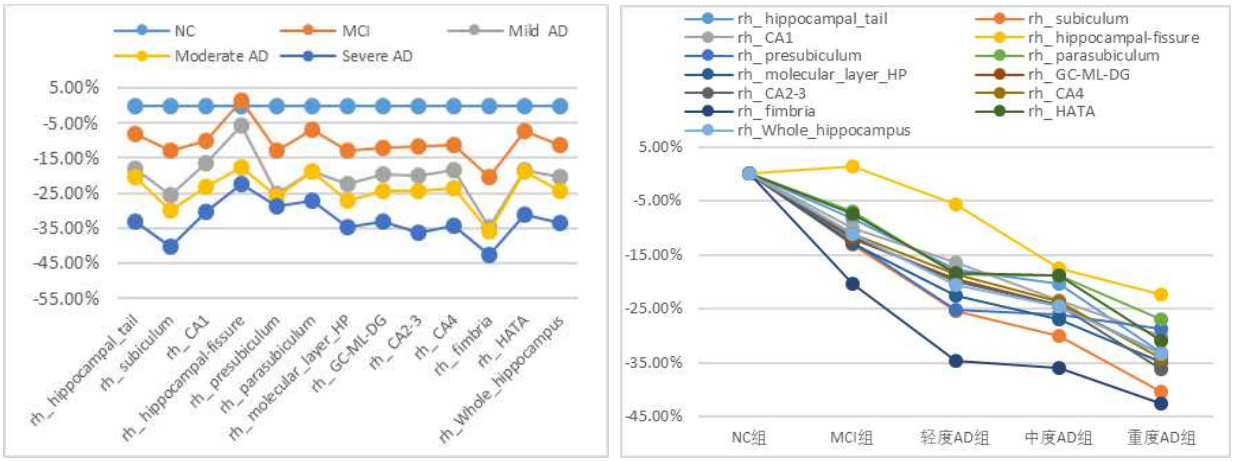
The atrophy rates of the average volumes of the whole hippocampus and hippocampal subfields on both of left and right sides at different stages of the disease compared with the NC group were showed in Figure 4. In each stage, the subiculum, presubiculum, molecular_layer_HP and fimbria showed higher atrophy rate than other hippocampal subfields, while the hippocampal-fissure and parasubiculum showed lower atrophy rate, especially on the left side of hippocampus.

3.4. Correlation between the whole hippocampus and hippocampal subfields volumes with cognitive function

It can be seen from Table 3 that in the NC group and the MCI group, there was no significant correlation of the bilateral whole hippocampus and hippocampal subfields volumes with the MMSE score (P<0.05). In the AD group (including Mild AD, Moderate AD and Severe AD group), the volumes of the left whole hippocampus and the hippocampal_tail, subiculum, CA1, molecular_layer_HP on the left side were all positively correlated with MMSE score (P<0.05). Analysis of all subjects' data showed that, in the entire course of disease from NC to Severe AD, except the bilateral parasubiculum, the other hippocampal subfileds and the whole hippocampal volume were all significantly positively correlated with MMSE scores (P<0.01).



(a) The left side



(b) The right side

Figure 4 Comparison of volume atrophy rates in hippocampal subfields between five groups

Table 3 Correlation between the whole hippocampus and hippocampal subfields volumes with cognitive function

	NC		MCI		AD (including Mild, Moderate and Severe AD)		All subjects	
	R	P	R	P	R	P	R	P
lh_Whole_hippocampus	0.027	0.874	0.223	0.190	0.240	0.049*	0.445	0.000**
rh_Whole_hippocampus	0.050	0.772	0.139	0.419	0.132	0.282	0.387	0.000**
lh_hippocampal_tail	0.060	0.730	0.174	0.331	0.286	0.018*	0.419	0.000**
rh_ hippocampal_tail	-0.043	0.802	0.153	0.373	0.214	0.080	0.379	0.000**
lh_subiculum	-0.024	0.854	0.164	0.339	0.323	0.007**	0.510	0.000**
rh_ subiculum	0.034	0.788	0.087	0.614	0.179	0.144	0.434	0.000**
lh_CA1	0.020	0.876	0.216	0.206	0.261	0.032*	0.397	0.000**
rh_ CA1	0.052	0.682	0.114	0.508	0.187	0.127	0.360	0.000**
lh_hippocampal-fissure	-0.013	0.921	0.044	0.801	0.216	0.078	0.305	0.000**
rh_ hippocampal-fissure	-0.033	0.848	0.097	0.575	0.207	0.090	0.280	0.001**
lh_presubiculum	-0.038	0.766	0.150	0.382	0.240	0.049*	0.459	0.000**
rh_ presubiculum	-0.009	0.944	0.039	0.823	0.009	0.945	0.300	0.000**
lh_parasubiculum	0.085	0.506	-0.049	0.777	0.038	0.756	0.186	0.029*
rh_ parasubiculum	0..081	0.638	-0.101	0.559	0.006	0.964	0.164	0.055
lh_molecular_layer_HP	-0.016	0.899	0.249	0.143	0.242	0.047*	0.454	0.000**
rh_ molecular_layer_HP	0.027	0.832	0.136	0.430	0.095	0.441	0.398	0.000**
lh_GC-ML-DG	0.027	0.832	0.290	0.086	0.162	0.188	0.399	0.000**
rh_ GC-ML-DG	0.034	0.788	0.186	0.278	0.191	0.120	0.383	0.000**
lh_CA2-3	0.013	0.921	0.262	0.123	0.132	0.285	0.365	0.000**
rh_ CA2-3	0.096	0.453	0.207	0.226	0.196	0.108	0.378	0.000**
lh_CA4	0.020	0.907	0.284	0.093	0.181	0.140	0.399	0.000**
rh_ CA4	0.017	0.922	0.178	0.299	0.214	0.079	0.392	0.000**
lh_fimbria	-0.067	0.601	0.357	0.033	-0.007	0.957	0.335	0.000**
rh_ fimbria	-0.045	0.724	0.236	0.165	-0.072	0.560	0.340	0.000*
lh_HATA	0.033	0.847	-0.016	0.925	0.120	0.328	0.309	0.000**
rh_ HATA	0.063	0.621	0.034	0.842	0.083	0.501	0.255	0.002**

'lh' represents the left hippocampus and hippocampal subfields, 'rh' represents the right hippocampus and hippocampal subfields.
The R value is Pearson's correlation coefficient, and the higher the R value is, the more relevant the two are. R>0 represents a positive correlation, and R<0 represents a negative correlation.
* indicates correlation, P<0.05. ** indicates a significant correlation, P<0.01.

4. Discussion and Conclutions

In recent years, some studies have shown that the hippocampal volume of patients with MCI and AD has different degrees of atrophy compared with normal people [28]. The results of this paper confirmed the same conclusion, and progressive atrophy of the whole hippocampus and hippocampal subfields in different stages of AD was recorded. The results of volume analysis showed that with the development of AD disease, the volume atrophy of each

hippocampal subfield showed an uneven trend. In addition, the correlation between hippocampal volume and MMSE score was analyzed, and the results showed that atrophy situation of different hippocampal subfields could reflect the severity of AD, which was correlated with the cognitive function decline of patients.

Firstly, we found that the whole hippocampus and most of the hippocampal subfields showed volume atrophy at the MCI stage, and showed significant volume atrophy at the Mild AD stage. It indicates that the hippocampus is a sensitive and vulnerable structure for AD patients, and whether the hippocampal volume is atrophic can be used as the basis for distinguishing normal people and patients [29]. As for Mild AD and MCI groups, there was only a statistically significant difference between the subiculum and presubiculum, and it was consistent with the findings of Carlesimo et al. [30]. Since MCI is a highly heterogeneous concept, rather than a disease entity, which has no specific case characteristics, the pathological changes of most MCI patients are similar to early or preclinical AD [31], so it is difficult to accurately classify AD and MCI. The results of this study indicate that the volume of the subiculum and presubiculum can be used as an effective means to distinguish AD and MCI, which is of certain clinical significance for the early diagnosis of AD.

In the three groups of Mild AD, Moderate AD and Severe AD, there was no statistical difference in the volume of the bilateral whole hippocampus, indicating that the whole hippocampal no longer shows significant atrophy in the middle and late stage of AD, so the volume of the whole hippocampus could not play an effective role in distinguishing the Mild AD, Moderate AD and Severe AD. It has been found that the hippocampal volume atrophy in AD patients has an accelerated phase in the early stage of the disease, and then decelerates in the later stage, so the hippocampal volume atrophy is in a S-shaped curve pattern [32]. This is because the pathogenesis of AD has a special time and spatial order. Not all brain structures are affected at the same time. In the earliest stage of AD, mainly the entorhinal cortex and other regions closely related to olfactory function are affected. With the pathological changes gradually, the hippocampus, medial temporal lobe and other regions were seriously affected. In the later period, it mainly affects the new cortex such as the prefrontal, parietal, and temporal lobe [33].

At the level of the hippocampal subfields, the volumes of the left subiculum, presubiculum, CA1, and molecular_layer_HP were still statistically different between Mild AD, Moderate AD and Severe AD group. It shows that the volume of these hippocampal subfields continues to significantly decrease during the whole development process of AD, which can play a certain role in identifying and monitoring the course of disease. Therefore, it is speculated that the disease development stages of AD patients can be classified based on the volumes of the left subiculum, presubiculum, CA1, and molecular_layer_HP.

In addition, at each stage of AD progression, the subiculum, presubiculum, CA1, molecular_layer_HP and fimbria showed higher atrophy rate than other subfields, indicating that these subregions were more sensitive to the development of disease than other regions and were more prone to change in volume. However, the hippocampal-fissure and parasubiculum showed lower atrophy rate at each stage, which proved that the volume of these two subfields were less affected by AD. For the differences in the volume atrophy of different hippocampal subfields, we speculate that this may be due to the different of cell and tissue structure in differences hippocampal subfields [34]. It may be also related to differences in the progression of pathological changes such as neuron loss and β-amyloid deposition in different hippocampal subfields in AD patients [35], as well as the structural and functional connection patterns of different hippocampal subfields are significantly different [36].

The result of correlation analysis showed that there was no significant correlation between the volume of whole hippocampal and hippocampal subfields and the MMSE score in the MCI group. The possible reason is that MCI is an intermediate stage between normal aging and AD, and the synaptic deletion mechanism in the normal development of the brain is in balance with the pathological mechanism of the disease, which leads to no correlation between the hippocampal volume and cognitive function of MCI patients [37].

In the AD group (including Mild AD, Moderate AD and Severe AD), only the left whole hippocampus and a small part of the hippocampal subfields were weakly correlated with MMSE scores. However, clinical manifestations and neuropsychological tests have shown that patients have a sustained and significant decline in cognitive function in the Mild AD to Moderate AD stage. Therefore, it is speculated that the hippocampal atrophy in the middle and late stage of AD patients is not the main cause of the decline of cognitive function, and the decline of cognitive function may be mainly caused by the shrinkage of gray matter in other brain structures [38].

By analyzing the data of all subjects, it can be seen that the whole hippocampus and all the hippocampal subfields except the parasubiculum were significantly positively correlated with the MMSE scores throughout the course of the disease, indicating that the atrophy of hippocampus and hippocampal subfields can reflect the degree of disease development in AD patients, so the volume of hippocampus and hippocampal subfields can be used as an effective means of AD prediction [39].

In addition, the degree of volume atrophy in the left hippocampus and hippocampal subfields is significantly correlated with the severity of AD than the right side, suggesting that the measurement of the left hippocampal

volume in AD patients can provide a higher reference value for clinical differential diagnosis [40]. For the left hippocampal susceptibility of AD patients, it is speculated that the reason may be related to the dominant hemisphere of human brain.

There are some limitations in this paper. Firstly, the volumes of hippocampus and hippocampal subfields were estimated only by structural MRI, and we should combine multimodal imaging data (such as structural MRI, functional MRI, PET) for further study. Secondly, the segmentation algorithm of hippocampus subfields in FreeSurfer6.0 requires high spatial resolution of images. Although the results of segmentation of each sample were visually examined, it was difficult to define the precise boundaries of different hippocampal subfields with 1.5t T1 images. In particular, estimation errors may occur in the results of small hippocampal subfields (<100 mm^3, such as parasubiculum, fimbria and HATA) or in very thin regions (such as molecular_layer_HP) [41]. In addition, this study is based on the analysis of the horizontal data, and it is worthwhile to obtain a large number of longitudinal data through follow-up investigation to further study the imaging characteristics such as the volumes of hippocampus and hippocampal subfields of AD and explore their correlations with cognitive function.

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Declarations

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Conflicts of interest: All of the authors have no conflict of interest to declare.

Ethics approval: This study was approved by the medical ethics committee of Chinese PLA General Hospital.

Consent to participate: All study participants have provided written informed consent.

Consent for publication: We have confirmed that a consent to publish was obtained.

Availability of data and material: All data of the study are real and available. The data of the NC, MCI, Mild AD and Moderate AD groups come from the ADNI dataset, and the subjects of Severe AD group are collected from Chinese PLA General Hospital.

Author contributions: Conception and study design (Kang G and Xu X), data collection or acquisition (Xu X, Li X, Han Y and Ling Z), statistical analysis (Kang G and Luo P), interpretation of results (Kang G, Xu X and Luo P), drafting the manuscript work or revising it critically for important intellectual content (Kang G, Xu X and Luo P) and approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (All authors).

References

[1] Bauer C M, Jara H, Killiany R, et al. Whole brain quantitative T2 MRI across multiple scanners with dual echo FSE: applications to AD, MCI, and normal aging[J]. Neuroimage, 2010, 52(2): 508-514.

[2] Braak H, Braak E V A. Staging of Alzheimer's disease-related neurofibrillary changes[J]. Neurobiology of aging, 1995, 16(3): 271-278.

[3] Markesbery W R. Neuropathologic alterations in mild cognitive impairment: a review[J]. Journal of Alzheimer's Disease, 2010, 19(1): 221-228.

[4] Tondelli M, Wilcock G K, Nichelli P, et al. Structural MRI changes detectable up to ten years before clinical Alzheimer's disease[J]. Neurobiology of aging, 2012, 33(4): 825. e25-825. e36.

[5] Evans M C, Barnes J, Nielsen C, et al. Volume changes in Alzheimer's disease and mild cognitive impairment: cognitive associations[J]. European radiology, 2010, 20(3): 674-682.

[6] Frisoni G B, Fox N C, Jack Jr C R, et al. The clinical use of structural MRI in Alzheimer disease[J]. Nature Reviews Neurology, 2010, 6(2): 67.

[7] Greene S J, Killiany R J, Alzheimer's Disease Neuroimaging Initiative. Hippocampal subregions are differentially affected in the progression to Alzheimer's disease[J]. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 2012, 295(1): 132-140.

[8] Drago V, Babiloni C, Bartrés-Faz D, et al. Disease tracking markers for Alzheimer's disease at the prodromal (MCI) stage[J]. Journal of Alzheimer's Disease, 2011, 26(s3): 159-199.

[9] Gosche K M, Mortimer J A, Smith C D, et al. Hippocampal volume as an index of Alzheimer neuropathology: findings from the Nun Study[J]. Neurology, 2002, 58(10): 1476-1482.

[10] Arnone D, McIntosh A M, Ebmeier K P, et al. Magnetic resonance imaging studies in unipolar depression: systematic review and meta-regression analyses[J]. European Neuropsychopharmacology, 2012, 22(1): 1-16.

[11] Coan A C, Kubota B, Bergo F P G, et al. 3T MRI quantification of hippocampal volume and signal in mesial temporal lobe epilepsy improves detection of hippocampal sclerosis[J]. American Journal of Neuroradiology, 2014, 35(1): 77-83.

[12] Aggleton J P. Multiple anatomical systems embedded within the primate medial temporal lobe: implications for hippocampal function[J]. Neuroscience & Biobehavioral Reviews, 2012, 36(7): 1579-1596.

[13] Maruszak A, Thuret S. Why looking at the whole hippocampus is not enough—a critical role for anteroposterior axis, subfield and activation analyses to enhance predictive value of hippocampal changes for Alzheimer's disease diagnosis[J]. Frontiers in cellular neuroscience, 2014, 8: 95.

[14] Small S A, Schobel S A, Buxton R B, et al. A pathophysiological framework of hippocampal dysfunction in ageing and disease[J]. Nature Reviews Neuroscience, 2011, 12(10): 585.

[15] Leandrou S, Mamais I, Petroudi S, et al. Hippocampal and entorhinal cortex volume changes in Alzheimer's disease patients and mild cognitive impairment subjects[C]//2018 IEEE EMBS International Conference on Biomedical & Health Informatics (BHI). IEEE, 2018: 235-238.

[16] Qian L, Liu R, Qin R, et al. The associated volumes of sub-cortical structures and cognitive domain in patients of Mild Cognitive Impairment[J]. Journal of Clinical Neuroscience, 2018, 56: 56-62.

[17] Peng G P, Feng Z, He F P, et al. Correlation of hippocampal volume and cognitive performances in patients with either mild cognitive impairment or Alzheimer's disease[J]. CNS neuroscience & therapeutics, 2015, 21(1): 15-22.

[18] Scelsi M A, Iglesias E, Schott J M, et al. THE ROLE OF HIPPOCAMPAL SUBFIELDS IN THE ATROPHY PROCESS IN ALZHEIMER'S DISEASE: AN IN-VIVO STUDY OF THE ADNI COHORT[J]. Alzheimer's & Dementia: The Journal of the Alzheimer's Association, 2017, 13(7): P788-P789.

[19] Yuyu X U, Qian X, Deng L, et al. MRI analysis of hippocampal head, body and tail volume changes in progresses of Alzheimer disease[J]. Chinese Journal of Medical Imaging Technology, 2017, 33(6): 853-858.

[20] Carlesimo G A, Piras F, Orfei M D, et al. Atrophy of presubiculum and subiculum is the earliest hippocampal anatomical marker of Alzheimer's disease[J]. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 2015, 1(1): 24-32.

[21] Müller-Ehrenberg L, Riphagen J M, Verhey F R J, et al. Alzheimer's Disease Biomarkers Have Distinct Associations with Specific Hippocampal Subfield Volumes[J]. Journal of Alzheimer's Disease, 2018 (Preprint): 1-13.

[22] Khan W, Westman E, Jones N, et al. Automated hippocampal subfield measures as predictors of conversion from mild cognitive impairment to Alzheimer's disease in two independent cohorts[J]. Brain topography, 2015, 28(5): 746-759.

[23] Folstein M F, Folstein S E, McHugh P R. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician[J]. Journal of psychiatric research, 1975, 12(3): 189-198.

[24] Morris J C. The Clinical Dementia Rating (CDR): current version and scoring rules[J]. Neurology, 1993.

[25] Arnone D, McIntosh A M, Ebmeier K P, et al. Magnetic resonance imaging studies in unipolar depression: systematic review and meta-regression analyses[J]. European Neuropsychopharmacology, 2012, 22(1): 1-16.

[26] Iglesias J E, Augustinack J C, 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[J]. Neuroimage, 2015, 115: 117-137.

[27] Iglesias J E, Van Leemput K, Augustinack J, et al. Bayesian longitudinal segmentation of hippocampal substructures in brain MRI using subject-specific atlases[J]. Neuroimage, 2016, 141: 542-555.

[28] Peng G P, Feng Z, He F P, et al. Correlation of hippocampal volume and cognitive performances in patients with either mild cognitive impairment or Alzheimer's disease[J]. CNS neuroscience & therapeutics, 2015, 21(1): 15-22.

[29] Dubois B, Feldman H H, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria[J]. The Lancet Neurology, 2014, 13(6): 614-629.

[30] Carlesimo G A, Piras F, Orfei M D, et al. Atrophy of presubiculum and subiculum is the earliest hippocampal anatomical marker of Alzheimer's disease[J]. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 2015, 1(1): 24-32.

[31] Haroutunian V, Hoffman L B, Been M S. Is there a neuropathology difference between mild cognitive impairment and dementia? [J]. Dialogues in clinical neuroscience, 2009, 11(2): 171.

[32] Sabuncu M R, Desikan R S, Sepulcre J, et al. The dynamics of cortical and hippocampal atrophy in Alzheimer disease[J]. Archives of neurology, 2011, 68(8): 1040-1048.

[33] Ji D X, Yin J Z. The spatio-temporal characteristics of diferent afected areas in the progression of Alzheimer disease pathology,imaging and clinical relevanc[J]. Chinese Journal of Clinicians (Electronic Edition) ,2013,7(24):11635-11638.

[34] Gu Y, Janoschka S, Ge S. Neurogenesis and hippocampal plasticity in adult brain[M]//Neurogenesis and Neural Plasticity. Springer, Berlin, Heidelberg, 2012: 31-48.

[35] Greene S J, Killiany R J, Alzheimer's Disease Neuroimaging Initiative. Hippocampal subregions are differentially affected in the progression to Alzheimer's disease[J]. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 2012, 295(1): 132-140.

[36] McCormick C, St-Laurent M, Ty A, et al. Functional and effective hippocampal–neocortical connectivity during construction and elaboration of autobiographical memory retrieval[J]. Cerebral cortex, 2013, 25(5): 1297-1305.

[37] Duarte A, Hayasaka S, Du A, et al. Volumetric correlates of memory and executive function in normal elderly, mild cognitive impairment and Alzheimer's disease[J]. Neuroscience letters, 2006, 406(1-2): 60-65.

[38] Smith A D. Imaging the progression of Alzheimer pathology through the brain[J]. Proceedings of the National Academy of Sciences, 2002, 99(7): 4135-4137.

[39] Eskildsen S F, Coupé P, Fonov V S, et al. Structural imaging biomarkers of Alzheimer's disease: predicting disease progression[J]. Neurobiology of aging, 2015, 36: S23-S31.

[40] Wachinger C, Salat D H, Weiner M, et al. Whole-brain analysis reveals increased neuroanatomical asymmetries in dementia for hippocampus and amygdala[J]. Brain, 2016, 139(12): 3253-3266.

[41] Mueller S G, Yushkevich P A, Das S, et al. Systematic comparison of different techniques to measure hippocampal subfield volumes in ADNI2[J]. NeuroImage: Clinical, 2018, 17: 1006-1018.

Figures

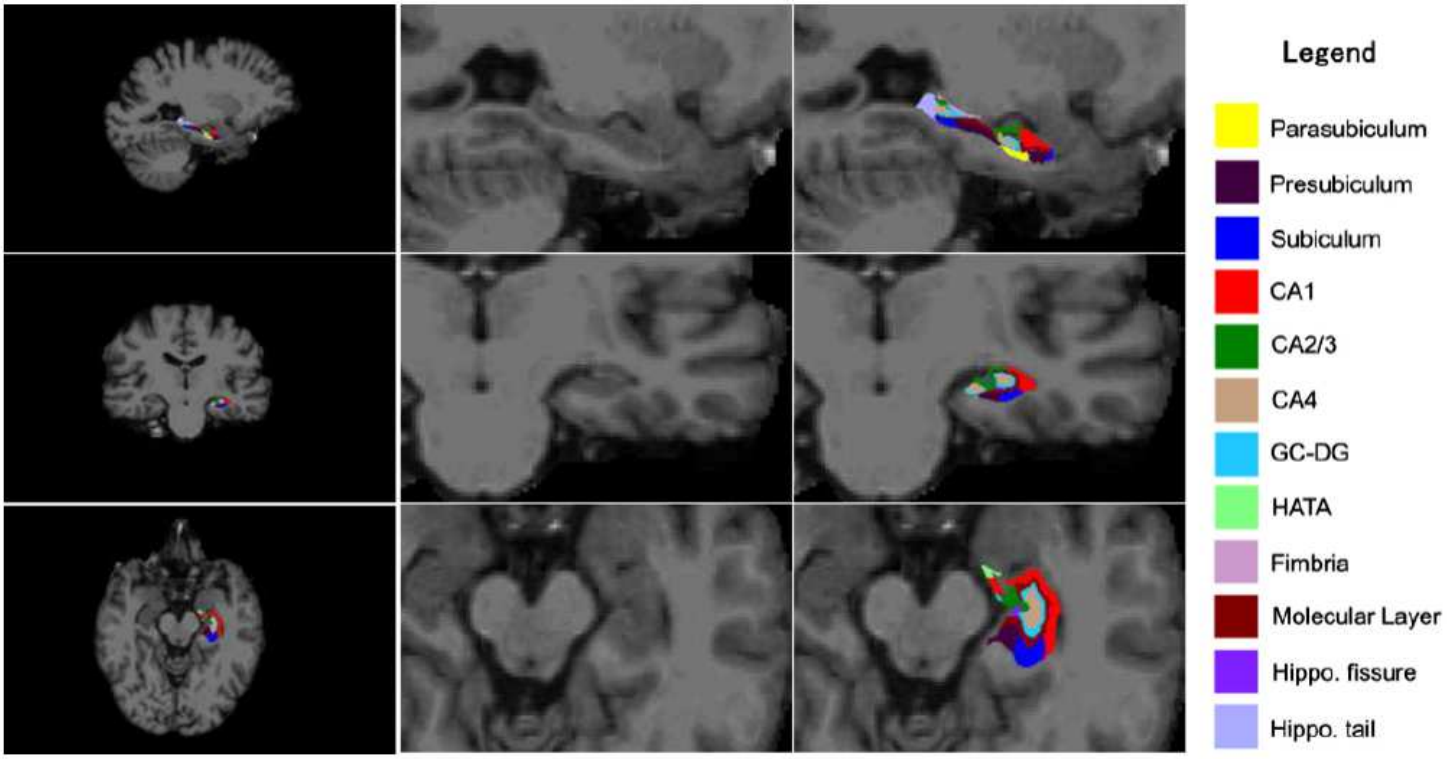


Figure 1

Segmentation of hippocampal subfields by Freesurfer 6.0

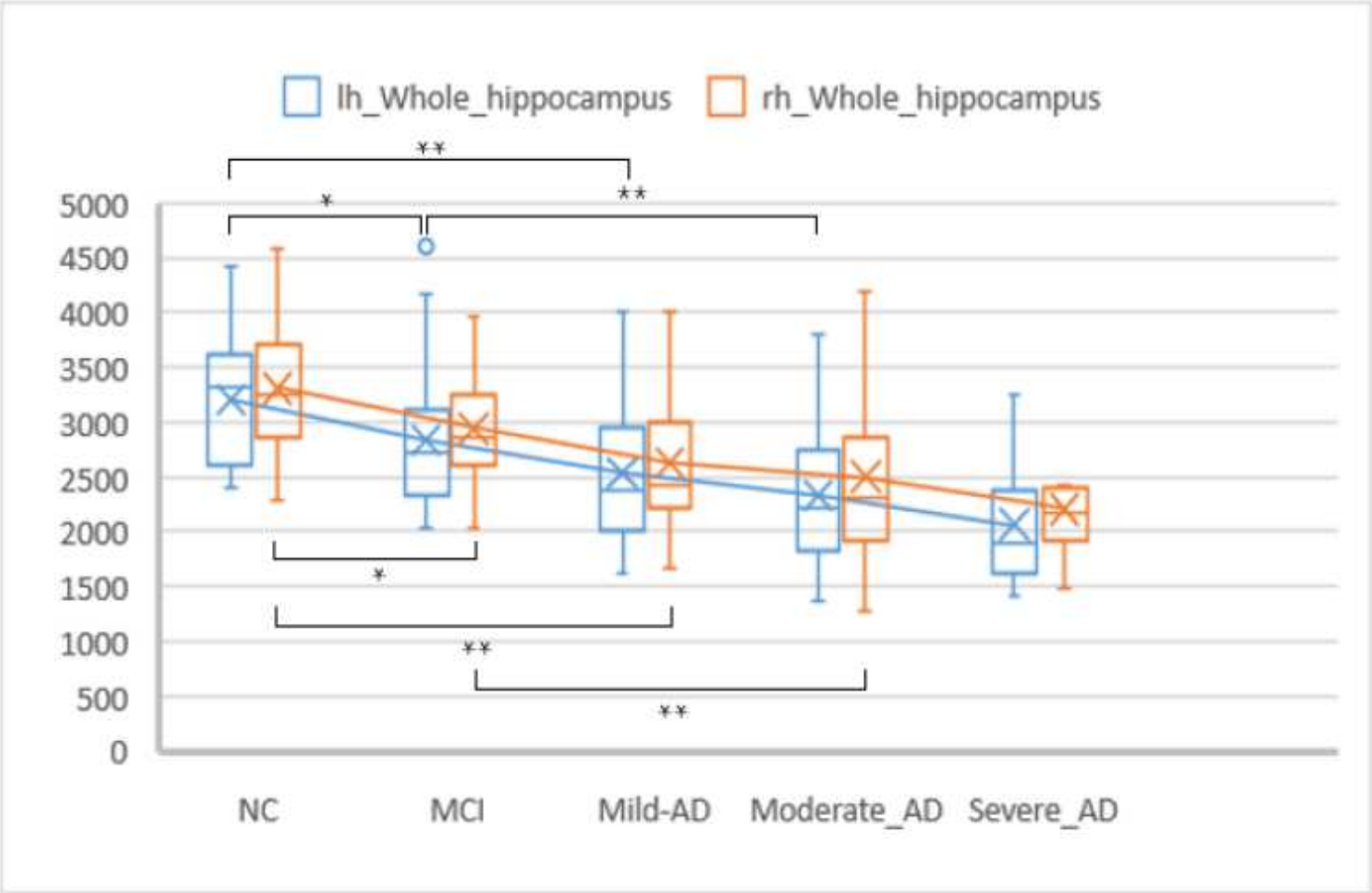


Figure 2

Comparison of the bilateral whole hippocampal volumes between five groups. *P<0.05，**P<0.01

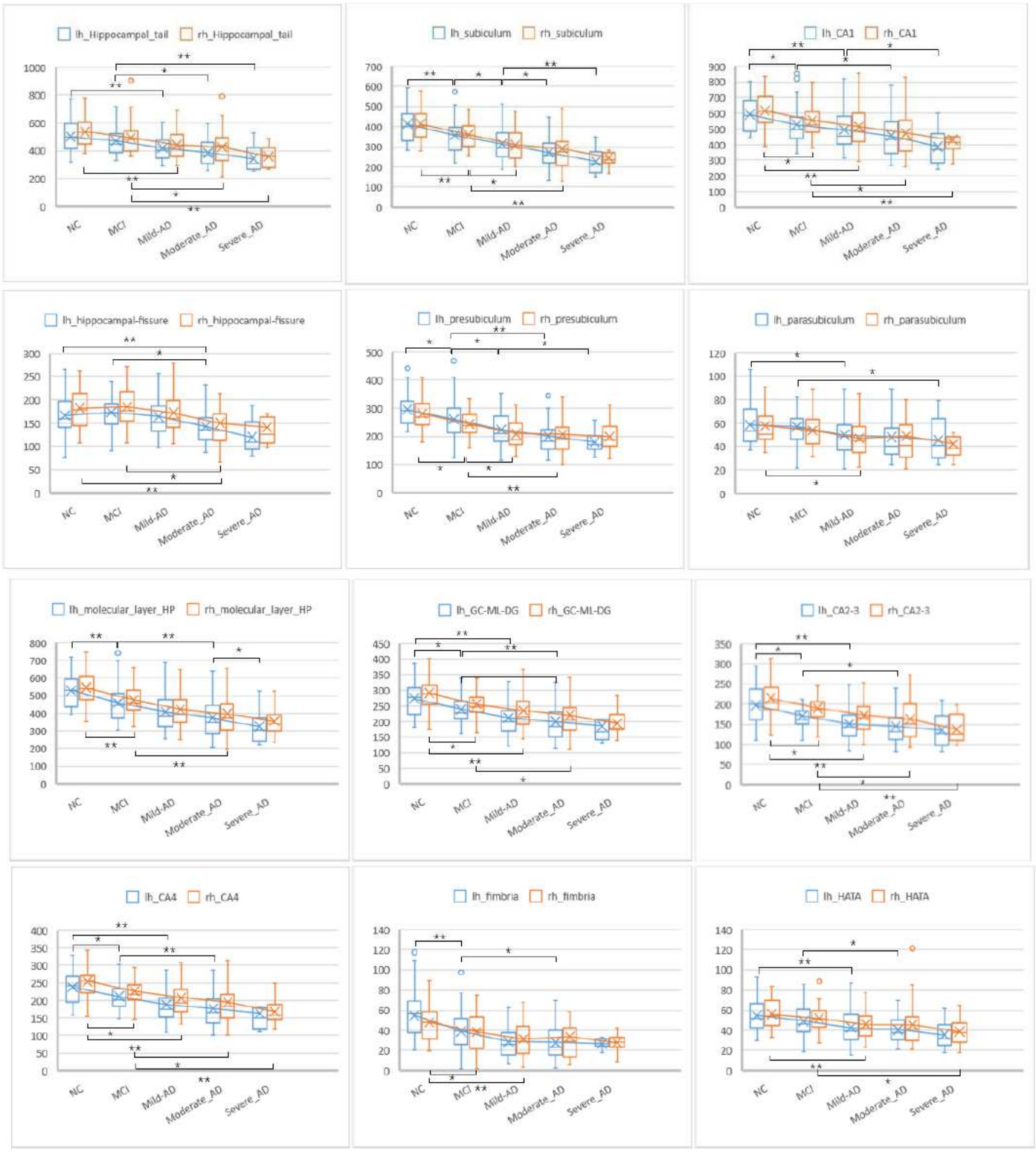
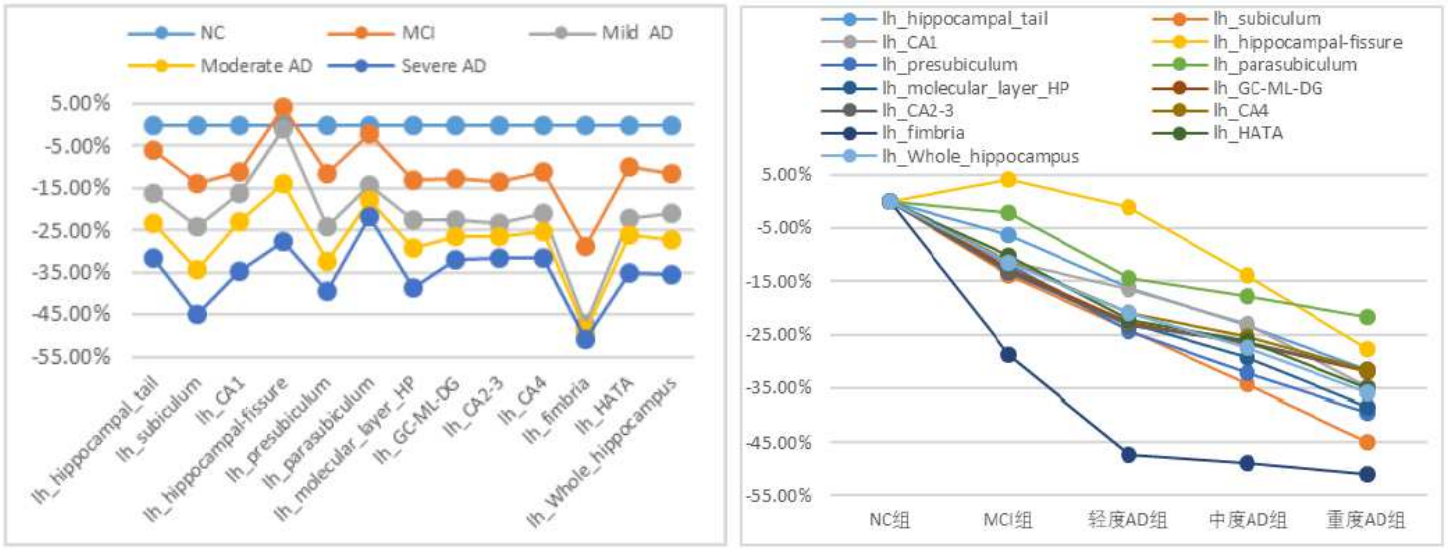
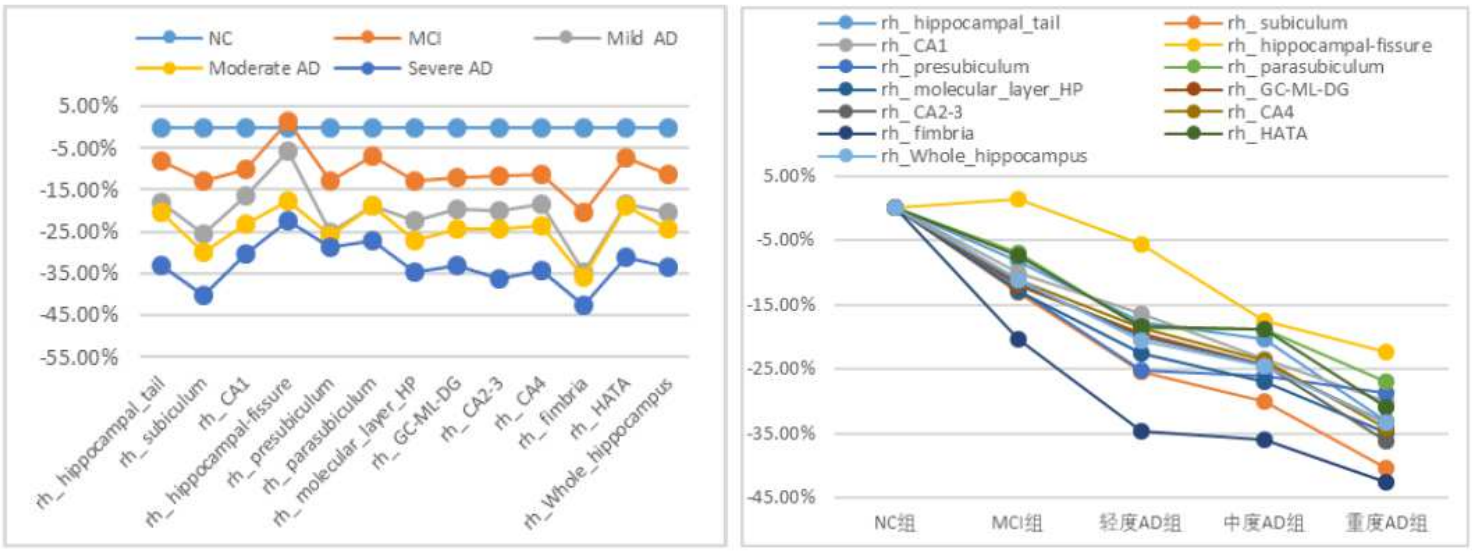


Figure 3

Comparison of hippocampal subfields volumes between five groups. *P<0.05，**P<0.01



(a) The left side



(b) The right side

Figure 4

Comparison of volume atrophy rates in hippocampal subfields between five groups

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