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Experimental study

Comparison of cortical and subcortical structural segmentation methods in Alzheimer's disease: A statistical approach

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ABSTRACT

Background: Automated segmentation methods are developed to help with the segmentation of different brain areas. However, their reliability has yet to be fully investigated. To have a more comprehensive understanding of the distribution of changes in Alzheimer's disease (AD), as well as investigating the reliability of different segmentation methods, in this study we compared volumes of cortical and subcortical brain segments, using HIPS, volBrain, CAT and BrainSuite automated segmentation methods between AD, mild cognitive impairment (MCI) and healthy controls (HC).

Methods: A total of 182 MRI images were taken from the minimal interval resonance imaging in Alzheimer's disease (MIRIAD; 22 AD and 22 HC) and the Alzheimer's disease neuroimaging initiative database (ADNI; 43 AD, 50 MCI and 45 HC) datasets. Statistical methods were used to compare different groups as well as the correlation between different methods.

Results: The two methods of volBrain and CAT showed a strong correlation (p's < 0.035 Bonferroni corrected for multiple comparisons). The two methods, however, showed no significant correlation with BrainSuite (p's $>$ 0.820 Bonferroni corrected). Furthermore, BrainSuite did not follow the same trend as the other three methods and only HIPS, volBrain and CAT showed strong conformity with the past literature with strong correlation with mini mental state examination (MMSE) scores.

Conclusion: Our results showed that automated segmentation methods HIPS, volBrain and CAT can be used in the classification of HC, AD and MCI. This is an indication that such methods can be used to inform researchers and clinicians of underlying mechanisms and progression of AD.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease, contributing to 60–70% of dementia cases [1] One important characteristic of AD is a significant loss of neurons and synapses, resulting in brain shrinkage and atrophy. Structural changes have been shown to be one of the earliest biomarkers that can be used in the diagnosis of AD, and mild cognitive impairment (MCI). Much effort has been devoted to find patterns of changes in the structure of different brain areas that can be reliably used for diagnosis of AD and MCI [2].

Earlier investigations relied mostly on manual segmentation of brain areas requiring a great deal of expertise and time. Therefore, the majority of the focus has been devoted to changes in the hippocampus due to its distinct structure. It has been shown that a loss in hippocampal volume can be an indication of AD. Further investigations have looked at subfields of the hippocampus, showing a nonuniform rate of neuroplasticity due to their specialisation [3]. For example, it has been shown that neurofibrillary tangle (NFT) begin in the medial temporal region and exhibit a characteristic distribution pattern across subfields, starting in the CA1 and later spreading to subiculum, CA2, CA3 and CA4/Dentate Gyrus [4].

With the development of semi- and fully-automated segmentation

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's disease neuroimaging initiative database; HC, healthy control; MCI, mild cognitive impairment; MIRIAD, minimal interval resonance imaging in Alzheimer's disease; MMSE, mini mental state examination.

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methods, however, it has now become easier and faster to segment not only the hippocampal area, but also other brain areas [5–9]. HIPpocampus subfield Segmentation (HIPS) [10] volBrain [11] Computational Anatomy Toolbox (CAT) [12–13] BrainSuite [14–15] and FreeSurfer [16]are some of the commonly used semi- and fully-automated methods. These methods, however, are still under development [17]. For example, CA1 segmentation in the FreeSurfer v5.3 was partially included in the subiculum [18] potentially explaining why the CA1 field was reported to be insensitive to AD pathology in some [19] but not all [20]. Similar findings have recently raised questions and concerns regarding the accuracy and consistency of these methods [21]. Therefore, it is important to investigate the accuracy of these methods further [22].

Benefiting from the computational power of automated methods, analysis of a large number of brain images has become more feasible. Large datasets of brain scans such as Minimal Interval Resonance Imaging in Alzheimer's Disease (MIRIAD) [23] and the Alzheimer's disease neuroimaging initiative database (ADNI) [24–25] public databases of Alzheimer's magnetic resonance imaging (MRI), offer a great opportunity to have a more comprehensive approach to the underlying mechanism and progression of AD. It also facilitates multisite studies to form a more accurate understanding of the disease.

Mini mental state examination (MMSE) is one of the commonly accepted measurements of cognitive ability, in particular in clinical settings. This measure has been widely used in classification of AD. For example, MIRIAD classifies participants with score between 12 and 26/30 as AD and those higher than 26/30 as healthy control (HC). There is huge body of literature showing correlation between MMSE score and brain atrophy [26].

The aim of this study was to investigate the reliability of four automated segmentation methods of volBrain, CAT and BrainSuite for segmentation of the whole brain, and HIPS for segmentation of subfields of hippocampus, which belongs to the same analysis tool as volBrain. We used images belonging to MIRIAD. Correlation of the volume of each brain area with MMSE scores are also investigated. To investigate the reliability of the three methods volBrain, CAT and BrainSuite, the correlation of their common brain areas is also reported.

2. Material and methods

2.1. Subjects

Our data analysis is based on data from 182 participants from two databases of Minimal Interval Resonance Imaging in Alzheimer's Disease (MIRIAD) (https://www.ucl.ac.uk/drc/research/research-methods/minimal-interval-resonance-imaging-alzheimers-disease-miriad) [23] and the Alzheimer's disease neuroimaging initiative database (ADNI) (http://adni.loni.usc.edu) [24–25]. For details of the demographics please see Supplementary Table 1.

2.2. Magnetic resonance imaging (MRI)

Data was extracted from MIRIAD, and ADNI databases. All of the MIRIAD subjects underwent MRI scanning on a 1.5 T Signa scanner (GE Medical Systems, Milwaukee, WI, USA). T1-weighted volumetric images were obtained using an inversion recovery prepared fast spoiled gradient echo sequence with acquisition parameters time to repetition = 15 ms, time to echo = 5.4 ms, flip angle = 15°, TI = 650 ms, a 24-cm field of view and a 256 × 256 matrix, to provide 124 contiguous 1.5-mm thick slices in the coronal plane (voxels 0.9735 × 0.9735 × 1.5 mm^3) [23]. Brain structural T1-weighted MRI data with 256 × 256 × 170 voxels and 1 × 1 × 1 mm^3 voxel size were extracted for ADNI subjects. ADNI data were obtained using an echo-planar imaging sequence on a 3 T Philips MRI scanner.

2.3. Methods

HIPS and volBrain; The volumes of Cerebrospinal fluid (CSF), white matter (WM), grey matter (GM), brain hemispheres, cerebellum and brainstem were obtained using volBrain pipeline [11]. This method is based on an advanced pipeline providing automatic segmentation of different brain structures from T1 weighted MRI, Supplementary Figure 1. The preprocessing is based on the following procedure: (1) a denoising step with an adaptive non-local mean filter, (2) an affine registration in the Montreal Neurological Institute (MNI) space, (3) a correction of the image inhomogeneities, and (4) an intensity normalisation. (5) Afterwards, MRI images are segmented in the MNI space using non-local patch-based multi-atlas method. Images were corrected for intensity inhomogeneity, and the images were segmented into brain/non-brain using a semi-automated technique (MIDAS). The non-local means filter was applied to each pixel of the image by computing a weighted average of surrounding pixels using a robust similarity measure that takes into account the neighbouring pixels surrounding the pixel being compared. This segmentation method is based on the idea of non-local patch-based label fusion technique, where patches of the brain image to be segmented are compared with those of the training library, looking for similar patterns within a defined search volume to assign the proper label. HIPS and volBrain are used for segmentation of the hippocampus subfields and the rest of the brain, respectively [10].

CAT; Computational Anatomy Toolbox (CAT) is a powerful package for brain T1-MRI data segmentation, Supplementary Figure 2. It is a voxel base estimation method. The CAT preprocessing steps are as follows: (1) spatial registration to a template, (2) tissue segmentation into grey, white matter and CSF, and (3) bias correction of intensity non-uniformities. (4) Finally, segments are extracted by scaling the amount of volume changes based on spatial registration, so that the total volume of grey matter in the modulated image remains the same as the original image. For correction of the orientation and size of the brain, non-linear registration methods are applied to the image. Projection-based thickness (PBT) method is used for calculation of the cortical thickness and central surface. Spatial-adaptive non-local means (SANLM) and classical Markov random field (MRF) were used for image denoising. Adaptive Maximum a Posterior (AMAP) method was used for segmentation.

BrainSuite; BrainSuite is an open-source software tool that enables largely automated cortical surface extraction from MRI of the brain, Supplementary Figure 3. BrainSuite includes automatic cortical surface extraction, bias field correlation, cerebrum labelling, and surface generation features. Also, this toolbox is used in tractography and connectivity matrix calculation in diffusion imaging data [14].

2.4. Statistical analysis

Independent-sample t-tests are run to compare the volume of different brain areas between the AD and HC, AD and MCI, and MCI and HC groups for volBrain, CAT and BrainSuite for the whole brain, and HIPS for the hippocampus subfields. Bivariate-correlation analyses are also run to investigate the relationship between volume and MMSE scores for all four segmentation methods. Correlational analyses are run between the common brain areas in volBrain, CAT and BrainSuite to investigate the relationship between the three methods. Bonferroni correction is applied to account for multiple comparison by reduction of the p threshold.

3. Results

Using three automatic segmentation methods CAT, volBrain and BrainSuite, we segmented the whole brain, and using HIPS we segmented the hippocampus. For details of the values for each of the segmentation methods, see Supplementary Data for ADNI and MIRIAD databases. Using independent-sample t-tests we compared the volumetric data for AD and HC, AD and MCI, and MCI and HC for each

segment. Supplementary Figures 4–7 show sample output images for one AD patient and one HC participant. Furthermore, we investigated the correlation of volumetric data with MMSE scores in AD and HC, AD and MCI, and MCI and HC groups.

CAT segmentation method returned data for 63 distinct brain areas. This method highlighted many brain areas that are significantly different between the AD and HC, AD and MCI, and MCI and HC groups, Table 1. In particular fusiform gyrus, parahippocampal gyrus, hippocampus, entorhinal cortex, amygdala, temporal gyri, thalamus, nucleus accumbens, insula, caudate and precuneus were significantly different. Importantly, the size of all these brain areas showed a strong correlation with MMSE scores. For further details see Supplementary Figures 8–10.

volBrain segmentation method returned data for eight distinct brain areas. In particular the amygdala, hippocampus, nucleus accumbens, thalamus and caudate were significantly different between the AD and HC, AD and MCI, and MCI and HC groups, Table 2. Again, the size of all these brain areas showed a strong correlation with MMSE scores. For further details see Supplementary Figure 11–13.

BrainSuite segmentation method returned data for 50 distinct brain areas. In contrast to CAT and volBrain, this method highlighted only six brain areas that are significantly different between the AD and HC, AD and MCI, and MCI and HC groups, Table 3. These brain areas included temporal gyri, third ventricle, supramarginal gyrus and angular gyrus. Similar to previous segmentation methods, all these brain areas showed strong correlation with MMSE scores. For further details see Supplementary Figures 14–16.

HIPS segmentation method returned data for the whole hippocampus and five of its subfields: CA1, CA2-CA3, CA4/Dentate Gyrus, Subiculum and strata radiatum/lacunosum/moleculare (SR-SL-SM). All these areas showed a significant difference between the AD and HC, AD and MCI, and MCI and HC groups, Table 4. The size of hippocampus and all its subfields showed strong correlation with MMSE scores. For further details see Supplementary Figure 17–19.

To investigate the relationship between the three whole-brain segmentation methods CAT, volBrain and BrainSuite, we ran correlational analysis, Table 5. Seven brain areas were common between these methods: nucleus accumbens, amygdala, caudate, globus pallidus, hippocampus, putamen and thalamus. CAT and volBrain showed strong correlation for nucleus accumbens, amygdala, caudate, hippocampus and thalamus. Two brain areas globus pallidus and putamen were not significantly correlated. These brain areas did not show significant difference between the AD and HC, AD and MCI, and MCI and HC groups either. BrainSuite, however, showed no significant correlation with either of the other two segmentation methods. For further details see Supplementary Figures 20–22.

4. Discussion

We used HIPS automated method to segment the subfields of hippocampus, and CAT, volBrain and BrainSuite automated methods to segment the whole brain using T1 weighted MRI data. Our results showed that all subfields of hippocampus in the Alzheimer's Disease (AD) and mild cognitive impairment (MCI) groups were significantly smaller than those of the healthy control (HC) group. The atrophy of all subcomponents of hippocampus were correlated with the MMSE measure. Quite a large portion of cortical and subcortical areas in the brain were also smaller in the AD, and MCI groups as compared to the control group, as evident from CAT and volBrain segmentation results. The shrinkage in these brain areas mostly showed a strong correlation with MMSE measure. BrainSuite failed to discriminate between the two groups. While CAT and volBrain shows a strong correlation, BrainSuite did not show any significant correlation with CAT and volBrain.

With the advancement of computational methods, fine-grain analysis of the brain areas is more feasible. Earlier methods relied heavily on manual segmentation of the brain areas, which was extremely time demanding and also required a great level of expertise. Therefore, the

Table 1
Summary of the independent-sample t-tests comparing volumetric data between different groups of the participants and the correlation of the data with MMSE scores using *CAT* method.

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
AD vs. HC MIRIAD					
Fusiform Gyrus	10.793	< 0.000001*	3.413	0.723	< 0.000001*
Parahippocampus Gyrus	9.936	< 0.000001*	3.142	0.536	< 0.000001*
Hippocampus	9.753	< 0.000001*	3.084	0.460	0.000001*
Entorhinal Cortex	9.717	< 0.000001*	3.073	0.476	< 0.000001*
Amygdala	9.043	< 0.000001*	2.860	0.445	0.000001*
Inferior Temporal Gyrus	8.939	< 0.000001*	2.827	0.653	< 0.000001*
Middle Temporal Gyrus	7.632	< 0.000001*	2.413	0.619	< 0.000001*
Temporal Pole	7.185	< 0.000001*	2.272	0.491	< 0.000001*
Basal Forebrain	6.658	< 0.000001*	2.105	0.453	0.000001*
Thalamus	6.344	< 0.000001*	2.006	0.471	0.000001*
Angular Gyrus	5.808	0.000001*	1.837	0.507	< 0.000001*
Accumbens	5.275	0.000005*	1.668	0.289	0.000236*
Inferior Occipital Gyrus	5.228	0.000006*	1.653	0.527	< 0.000001*
Superior Temporal Gyrus	5.186	0.000007*	1.640	0.513	< 0.000001*
Supramarginal Gyrus	5.101	0.000009*	1.613	0.498	< 0.000001*
Anterior Insula	4.955	0.000014*	1.567	0.346	0.000043*
Occipital Fusiform Gyrus	4.519	0.000054*	1.429	0.472	0.000001*
Middle Occipital Gyrus	4.515	0.000055*	1.428	0.417	0.000004*
Posterior Insula	4.447	0.000068*	1.406	0.338	0.000054*
Planum Polare	4.232	0.000131*	1.338	0.349	0.000038*
Anterior Cingulate Gyrus	4.225	0.000134*	1.336	0.362	0.000025*
Superior Parietal Lobule	4.131	0.000179*	1.306	0.423	0.000003*
Caudate	3.959	0.000301*	1.252	0.234	0.001155
Subcallosal Area	3.847	0.000420*	1.217	0.299	0.000180*
Middle Frontal Gyrus	3.796	0.000490*	1.200	0.328	0.000073*
Medial Orbital Gyrus	3.739	0.000579*	1.182	0.278	0.000331*
Inferior Frontal Gyrus	3.691	0.000666*	1.167	0.373	0.000017*
Precuneus	3.633	0.000788*	1.149	0.349	0.000038*
Superior Medial Frontal Gyrus	3.543	0.001023	1.120	0.312	0.000120*
Putamen	3.525	0.001077	1.115	0.203	0.002822
Temporal	3.489	0.001195	1.103	0.340	0.000051*
Anterior Orbital Gyrus	3.413	0.001484	1.079	0.244	0.000894
Posterior Orbital Gyrus	3.272	0.002203	1.035	0.255	0.000643*
Lingual Gyrus	3.263	0.002262	1.032	0.304	0.000158*
Posterior Cingulate Gyrus	3.226	0.002506	1.020	0.314	0.000117*
Central Operculum	3.116	0.003388	0.985	0.372	0.000018*
Frontal Operculum	2.999	0.004638	0.948	0.265	0.000479*
Supplementary Motor Cortex	2.969	0.005032	0.939	0.310	0.000128*
Exterior Cerebellum	2.934	0.005513	0.928	0.163	0.007986
Superior Frontal Gyrus	2.870	0.006523	0.908	0.199	0.003066
Parietal Operculum	2.864	0.006637	0.906	0.320	0.000095*
Middle Cingulate Gyrus	2.734	0.009272	0.865	0.269	0.000432*
Gyrus Rectus	2.660	0.011190	0.841	0.143	0.013603

(*continued on next page*)

Table 1 (*continued*)

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
Optic Chiasm	2.531	0.015401	0.800	0.066	0.101456
Lateral Orbital Gyrus	2.427	0.019841	0.767	0.158	0.009194
Temporal Transverse Gyrus	2.345	0.024056	0.742	0.189	0.003960
Superior Occipital Gyrus	2.290	0.027347	0.724	0.245	0.000849
Medial Precentral Gyrus	2.250	0.030015	0.712	0.196	0.003321
Cuneus	2.091	0.042968	0.661	0.187	0.004135
Inferior Frontal Orbital Gyrus	2.063	0.045679	0.652	0.123	0.022887
Postcentral Gyrus	1.687	0.099402	0.533	0.142	0.013747
Frontal Pole	1.603	0.116854	0.507	0.067	0.098513
Occipital Pole	1.596	0.118311	0.505	0.127	0.020199
Inferior Frontal Angular Gyrus	1.518	0.136864	0.480	0.134	0.017041
Cerebellum White Matter	1.319	0.194795	0.417	0.079	0.071875
Precentral Gyrus	1.196	0.238644	0.378	0.124	0.022441
Medial Postcentral Gyrus	1.061	0.294904	0.336	0.070	0.090174
Brainstem	−0.875	0.386852	0.277	0.005	0.640175
Cerebellar Vermal Lobules VI-VII	0.699	0.488487	0.221	0.024	0.325448
Cerebellar Vermal Lobules VIII-X	0.617	0.541029	0.195	0.015	0.434774
Cerebellar Vermal Lobules I-V	0.218	0.828189	0.069	0.016	0.430517
Globus Pallidus	−0.212	0.832833	0.067	0.001	0.860265
Calcarine Cortex	0.198	0.843964	0.063	0.013	0.473158
AD vs. HC ADNI					
Hippocampus	9.404	< 0.000001*	3.084	0.419	0.0000494*
Amygdala	8.497	< 0.000001*	3.073	0.594	< 0.000001*
Entorhinal Area	8.098	< 0.000001*	2.860	−0.207	0.0529441
Inferior Temporal Gyrus	6.774	< 0.000001*	2.827	0.183	0.0871786
Parahippocampus Gyrus	6.594	< 0.000001*	2.413	0.101	0.3491747
Temporal Pole	6.437	< 0.000001*	2.272	−0.012	0.9144256
Fusiform Gyrus	6.309	< 0.000001*	2.105	0.193	0.0714092
Middle Temporal Gyrus	6.259	< 0.000001*	2.006	0.122	0.2593355
Third Ventricle	5.525	< 0.000001*	1.837	0.684	< 0.000001*
Thalamus Proper	4.831	0.000005*	1.668	0.363	0.0005046*
Superior Temporal Gyrus	4.282	0.00004*	1.653	0.016	0.8795689
Supramarginal Gyrus	4.009	0.00012*	1.640	−0.031	0.7757335
Angular Gyrus	3/353	0/00118*	1.428	0.229	0.0315600
Basal Cerebrum and Forebrain Brain	3.764	0/00030*	1.613	0.245	0.0211458
Middle Occipital Gyrus	3.629	0/00048*	1.567	0.165	0.1247199
Accumbens	3.462	0/00083*	1.429	0.629	< 0.000001*
Inferior Occipital Gyrus	3.288	0.00146*	1.406	0.187	0.0818532
Temporal	3.208	0.00187*	1.338	−0.043	0.6915130
Inferior Frontal Gyrus	2.790	0.006485	1.336	0.115	0.2876243
Superior Parietal Lobule	2.769	0.006886	1.306	−0.017	0.8752262
Superior Frontal Gyrus	2.708	0.008158	1.252	0.036	0.7381613
Posterior Cingulate Gyrus	2.583	0.011481	1.217	0.107	0.3217183

Table 1 (*continued*)

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
Caudate	2.432	0.017087	1.182	0.543	< 0.000001*
Planum Polare	2.432	0.017080	1.200	0.047	0.6648243
Anterior Insula	2.387	0.019158	1.167	0.234	0.0283361
Superior Occipital Gyrus	2.339	0.021611	1.149	−0.018	0.8679229
Anterior Cingulate Gyrus	2.266	0.025943	1.120	0.237	0.0262514
Posterior Orbital Gyrus	2.236	0.027924	1.115	−0.052	0.6326872
Middle Frontal Gyrus	2.176	0.032294	1.103	0.169	0.1146428
Precuneus	2.171	0.032637	1.079	−0.102	0.3419842
Occipital Fusiform Gyrus	2.161	0.033434	1.035	0.119	0.2689258
Temporal Transverse Gyrus	2.158	0.033682	1.032	−0.004	0.9696884
Lateral Ventricle	2.146	0.034612	1.020	0.380	0.0002562*
Cerebral White Matter	−2.140	0.037390	0.985	0.476	0.0000028*
Posterior Insula	1.989	0.049787	0.948	0.100	0.3531347
Medial Orbital Gyrus	1.913	0.058997	0.939	0.143	0.1837937
Ventral Ventricle	1.911	0.059321	0.928	0.333	0.0015289*
Cerebrum and Motor	1.896	0.061245	0.908	−0.032	0.7645920
Putamen	1.875	0.064159	0.906	0.365	0.0004706*
Parietal Operculum	1.871	0.064695	0.865	0.070	0.5157279
Medial Frontal Cerebrum	1.867	0.065282	0.841	0.174	0.1058410
Precentral Gyrus	1.860	0.066161	0.800	0.044	0.6825811
Postcentral Gyrus	1.833	0.070238	0.767	0.070	0.5196872
Inferior Lateral Ventricle	1.797	0.075787	0.742	0.396	0.0001355*
Frontal Operculum	1.748	0.084022	0.724	0.202	0.0588634
Subcallosal Area	1.739	0.085438	0.712	−0.037	0.7324943
Central Operculum	1.724	0.088258	0.661	0.219	0.0406681
Gyrus Rectus	1.656	0.101231	0.652	0.192	0.0725703
Lingual Gyrus	1.606	0.111795	0.533	0.180	0.0928955
Cerebellum White Matter	−1.605	0.112006	0.507	0.516	0.0000003*
CSF	−1.511	0.134440	0.505	0.435	0.0000226*
Occipital Pole	1.475	0.143775	0.480	0.120	0.2635417
Inferior Frontal Angular Gyrus	1.465	0.146313	0.417	0.010	0.9298917
Optic Chiasm	1.453	0.149599	0.378	0.292	0.0058490*
Superior Medial Frontal Gyrus	1.426	0.157436	0.336	0.128	0.2328524
Cuneus	1.403	0.164011	0.277	0.215	0.0440727
Lateral Orbital Gyrus	1.211	0.228967	0.221	0.178	0.0970858
Medial Precentral Gyrus	1.054	0.294516	0.195	−0.135	0.2099596
Anterior Orbital Gyrus	0.965	0.337243	0.069	0.232	0.0293916
Brainstem	−0.932	0.353515	0.067	0.567	< 0.000001*
Calcarine and Cerebrum	0.861	0.391110	0.063	0.228	0.0329128
Cerebellar Lobules I-V	−0.823	0.412626	0.174	0.261	0.0139193
Inferior Frontal Orbital Gyrus	0.702	0.484444	0.148	0.109	0.3099968
Middle Cingulate Gyrus	0.676	0.500429	0.143	0.174	0.1048655
Medial Postcentral Gyrus	0.587	0.558602	0.124	0.140	0.1925617
Cerebellar Lobules VIII-X	−0.349	0.727200	0.074	0.254	0.0171581
Fourth Ventricle	−0.311	0.756213	0.066	0.663	< 0.000001*
Pallidum	−0.242	0.809132	0.051	0.376	0.0003026*
Frontal Pole	−0.237	0.812977	0.050	0.194	0.0706686
Exterior Cerebellum	−0.219	0.826609	0.046	0.537	< 0.000001*
Cerebellar Lobules VI-VII	0.000	0.999546	0.001	0.261	0.0141165

(*continued on next page*)

Table 1 (*continued*)

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
MCI vs. AD ADNI					
Inferior Temporal Gyrus	−3.256	0.001586	3.084	0.115	0.271879
Hippocampus	−3.034	0.003144	3.073	0.297	0.003824*
Amygdala	−2.926	0.004323	2.860	0.375	0.0002171*
Fusiform Gyrus	−2.904	0.004622	2.827	0.133	0.202203
Middle Temporal Gyrus	−2.679	0.008745	2.413	0.069	0.513523
Caudate	−2.537	0.012862	2.272	0.325	0.001483*
Inferior Occipital Gyrus	−2.525	0.013272	2.105	−0.122	0.244229
Middle Occipital Gyrus	−2.477	0.015075	2.006	0.095	0.363967
Entorhinal Area	−2.449	0.016205	1.837	0.152	0.144564
Parahippocampus Gyrus	−2.336	0.021682	1.668	0.056	0.592109
Superior Parietal Lobule	−2.231	0.028075	1.653	0.015	0.888521
Angular Gyrus	−2.208	0.029708	1.640	0.176	0.091302
Accumbens	−2.155	0.033797	1.613	0.376	0.0002041*
Brainstem	−2.028	0.045470	1.429	0.340	0.000857*
Supramarginal Gyrus	−2.028	0.045456	1.567	−0.020	0.849047
Occipital Pole	−1.942	0.055169	1.428	0.067	0.520334
Fourth Ventricle	−1.870	0.064603	1.406	0.440	0.000010*
Posterior Cingulate Gyrus	−1.845	0.068209	1.338	0.059	0.575217
Occipital Fusiform Gyrus	−1.818	0.072301	1.336	−0.065	0.538476
Superior Occipital Gyrus	−1.793	0.076284	1.306	0.018	0.865996
Precuneus	−1.700	0.092469	1.252	0.058	0.583300
Temporal Pole	−1.669	0.098399	1.217	0.010	0.927080
Lateral Ventricle	−1.548	0.124984	1.200	0.246	0.017391
Ventral Ventricle	−1.514	0.133308	1.182	0.211	0.042073
Precentral Gyrus	1.389	0.167942	1.167	−0.029	0.784291
Third Ventricle	−1.366	0.175057	1.149	0.441	< 0.000001*
Temporal Transverse Gyrus	−1.334	0.185358	1.120	−0.003	0.973923
Parietal Operculum	−1.297	0.197861	1.103	−0.055	0.602180
Planum Polare	−1.297	0.197600	1.115	0.037	0.723224
Cerebellar Lobules VIII-X	−1.255	0.212658	1.079	0.193	0.063884
Anterior Insula	−1.242	0.217097	1.035	0.185	0.075645
Medial Orbital Gyrus	−1.237	0.219021	1.032	0.093	0.376657
Middle Cingulate Gyrus	−1.227	0.222921	1.020	0.103	0.324278
Cerebellar Lobules VI-VII	−1.224	0.223748	0.985	0.204	0.049898
Exterior Cerebellum	−1.206	0.230916	0.948	0.322	0.001672*
Basal Cerebrum and Forebrain Brain	−1.182	0.239930	0.939	−0.191	0.066070
Putamen	−1.167	0.246009	0.928	0.217	0.036283
Superior Temporal Gyrus	−1.126	0.262845	0.908	0.013	0.898857
Anterior Cingulate Gyrus	−1.023	0.308867	0.906	0.189	0.069717
Cerebellar Lobules I-V	−0.986	0.326261	0.865	0.204	0.049268
Cuneus	−0.935	0.352259	0.841	0.159	0.129108
Posterior Insula	−0.876	0.383196	0.800	0.056	0.594816
Subcallosal Area	−0.853	0.395748	0.767	−0.027	0.800205
Medial Frontal Cerebrum	−0.833	0.406795	0.742	0.103	0.325051
Medial Precentral Gyrus	0.830	0.408420	0.724	0.079	0.450841
Thalamus Proper	−0.828	0.409548	0.712	0.212	0.041373
Posterior Orbital Gyrus	−0.825	0.411357	0.661	0.044	0.674969
Temporal	−0.798	0.426750	0.652	0.029	0.784944
Pallidum	−0.744	0.458265	0.533	0.244	0.018630
	0.734	0.464672	0.507	0.061	0.561631
Inferior Frontal Orbital Gyrus					
Postcentral Gyrus	−0.676	0.500650	0.505	0.051	0.627363
Gyrus Rectus	−0.650	0.517122	0.480	0.128	0.220615
Cerebellum White Matter	−0.647	0.519174	0.417	0.307	0.002791
Inferior Lateral Ventricle	−0.606	0.545505	0.378	0.248	0.016371
Anterior Orbital Gyrus	−0.590	0.556529	0.336	0.177	0.089671
Lingual Gyrus	−0.539	0.591088	0.277	0.112	0.284682
Calcarine and Cerebrum	−0.479	0.633015	0.221	0.165	0.113855
Frontal Pole	0.336	0.737406	0.195	0.142	0.175570
Cerebrum and Motor	0.292	0.770866	0.069	−0.020	0.848462
Inferior Frontal Angular Gyrus	0.289	0.772710	0.067	0.004	0.967000
Frontal Operculum	−0.262	0.793898	0.063	0.150	0.150335
CSF	−0.223	0.823972	0.047	0.302	0.003296
Superior Frontal Gyrus	0.142	0.887139	0.03	0.024	0.817511
Lateral Orbital Gyrus	−0.132	0.894760	0.028	0.109	0.296671
Inferior Frontal Gyrus	0.126	0.899630	0.027	0.062	0.556217
Medial Postcentral Gyrus	0.118	0.905977	0.025	−0.086	0.414002
Central Operculum	0.113	0.909873	0.024	−0.160	0.125628
Cerebral White Matter	0.106	0.915576	0.022	0.307	0.002793
Middle Frontal Gyrus	−0.086	0.931368	0.018	0.103	0.326771
Superior Medial Frontal Gyrus	0.068	0.945569	0.014	0.073	0.485031
Optic Chiasm	−0.067	0.946404	0.014	0.211	0.042460
MCI vs. HC ADNI					
Hippocampus	6.571	< 0.000001*	3.084	0.338	0.000794
Amygdala	5.736	< 0.000001*	3.073	0.441	0.000008
Entorhinal Area	5.621	< 0.000001*	2.860	−0.194	0.058938
Parahippocampus Gyrus	4.856	0.000004*	2.827	0.087	0.400465
Third Ventricle	4.286	0.000044*	2.413	0.498	0.000000
Temporal Pole	4.090	0.000091*	2.272	−0.019	0.854344
Thalamus Proper	3.641	0.000446*	2.105	0.257	0.011921
Precentral Gyrus	3.595	0.000520*	2.006	0.066	0.528051
Fusiform Gyrus	3.269	0.001513*	1.837	0.166	0.108938
Brainstem	−3.258	0.001565*	1.668	0.400	0.000059
Inferior Temporal Gyrus	3.185	0.001969*	1.653	−0.155	0.132531
Superior Temporal Gyrus	3.072	0.002783*	1.640	0.021	0.839452
Middle Temporal Gyrus	2.964	0.003847*	1.613	0.123	0.236521
Inferior Frontal Gyrus	2.898	0.004670*	1.567	0.108	0.296527
Superior Frontal Gyrus	2.872	0.005044*	1.429	0.032	0.758188
Cerebellum White Matter	−2.707	0.008056*	1.428	0.361	0.000325
Temporal	2.543	0.012638	1.406	0.060	0.566552
Basal Cerebrum and Forebrain Brain	2.403	0.018205	1.338	0.221	0.031477
Medial Precentral Gyrus	2.389	0.018882	1.336	0.123	0.234996
Cerebral White Matter	−2.300	0.023651	1.306	0.347	0.000570
Fourth Ventricle	−2.167	0.032723	1.252	0.492	0.000000
Middle Frontal Gyrus	2.120	0.036658	1.217	0.138	0.181354
Cerebrum and Motor	2.113	0.037200	1.200	0.026	0.802510

(*continued on next page*)

Table 1 (*continued*)

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
Inferior Frontal Angular Gyrus	2.018	0.046397	1.182	−0.016	0.878148
CSF	−1.913	0.058814	1.167	0.347	0.000582
Central Operculum	1.839	0.068976	1.149	−0.205	0.046610
Cerebellar Lobules VIII-X	−1.773	0.079347	1.120	0.221	0.031240
Cerebellar Lobules I-V	−1.709	0.090765	1.115	0.228	0.026165
Accumbens	1.683	0.095719	1.103	0.477	0.000001
Inferior Frontal Orbital Gyrus	1.546	0.125314	1.079	0.106	0.306983
Exterior Cerebellum	−1.530	0.129384	1.035	0.366	0.000261
Supramarginal Gyrus	1.523	0.131053	1.032	−0.025	0.812476
Frontal Operculum	1.496	0.137797	1.020	0.180	0.081044
Superior Medial Frontal Gyrus	1.469	0.144987	0.985	0.123	0.235821
Optic Chiasm	1.442	0.152424	0.948	0.238	0.020176
Inferior Lateral Ventricle	1.366	0.175196	0.939	0.310	0.002268
Posterior Orbital Gyrus	1.336	0.184783	0.928	0.067	0.519365
Postcentral Gyrus	1.239	0.218444	0.908	0.069	0.504971
Lingual Gyrus	1.218	0.226054	0.906	0.152	0.142482
Planum Polare	1.159	0.249380	0.865	−0.066	0.527619
Cerebellar Lobules VI-VII	−1.151	0.252304	0.841	0.227	0.027142
Pallidum	−1.091	0.277866	0.800	0.272	0.007734
Lateral Orbital Gyrus	1.062	0.290926	0.767	0.151	0.143033
Gyrus Rectus	1.051	0.295649	0.742	0.161	0.119422
Anterior Cingulate Gyrus	0.975	0.331716	0.724	0.218	0.033446
Medial Frontal Cerebrum	0.910	0.365097	0.712	0.139	0.179694
Temporal Transverse Gyrus	0.907	0.366689	0.661	−0.014	0.892777
Anterior Insula	0.873	0.384793	0.652	0.216	0.035135
Subcallosal Area	0.834	0.406411	0.533	0.040	0.699129
Putamen	0.810	0.419480	0.507	0.267	0.008925
Posterior Insula	0.795	0.428489	0.505	0.074	0.474955
Medial Postcentral Gyrus	0.778	0.437963	0.480	0.126	0.224334
Inferior Occipital Gyrus	0.754	0.452613	0.417	0.160	0.121425
Middle Occipital Gyrus	0.717	0.474877	0.378	0.138	0.183365
Middle Cingulate Gyrus	−0.708	0.480102	0.336	0.141	0.172537
Angular Gyrus	0.703	0.483450	0.277	0.214	0.037005
Posterior Cingulate Gyrus	0.651	0.516299	0.221	0.105	0.309007
Lateral Ventricle	0.622	0.535027	0.195	0.287	0.004807
Superior Occipital Gyrus	0.617	0.538210	0.069	−0.024	0.820848
Cuneus	0.597	0.551708	0.067	0.202	0.049658
Medial Orbital Gyrus	0.542	0.588513	0.063	0.132	0.202920
Occipital Pole	−0.534	0.594046	0.113	0.118	0.253082
Superior Parietal Lobule	0.442	0.659057	0.093	0.023	0.828197
Ventral Ventricle	0.432	0.666269	0.091	0.247	0.015688
Calcarine and Cerebrum	0.411	0.681960	0.087	0.210	0.041297
Occipital Fusiform Gyrus	0.382	0.703058	0.081	0.109	0.293014
Anterior Orbital Gyrus	0.317	0.751715	0.067	0.215	0.036029
Precuneus	0.304	0.761611	0.064	−0.092	0.375141
Parietal Operculum	0.189	0.850138	0.040	0.073	0.484076
Frontal Pole	0.155	0.877003	0.033	0.175	0.090362
Caudate	−0.111	0.911414	0.023	0.395	0.000074

Notes: ↑ rows are sorted based on the *p* values for the *t*-test; * $p < 0.000793$ Bonferroni corrected for multiple comparison; *d* represents Cohen's d effect size; MMSE: mini mental state examination.

Table 2
Summary of the independent-sample t-tests comparing volumetric data between different groups of the participants and the correlation of the data with MMSE scores using *volBrain* method.

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
AD vs. HC MIRIAD					
Amygdala	10.217	< 0.000001*	3.231	0.428	0.000001*
Hippocampus	6.58	< 0.000001*	2.081	0.256	0.000395*
Accumbens	5.813	0.000001*	1.838	0.339	0.000027*
Thalamus	4.422	0.000065*	1.398	0.317	0.000057*
Caudate	4.149	0.000154*	1.312	0.169	0.005091
Cerebellum	2.063	0.045216	0.652	0.094	0.041135
Globus Pallidus	−1.103	0.276245	0.349	0.026	0.287427
Putamen	0.846	0.402030	0.268	0.019	0.366267
AD vs. HC ADNI					
Hippocampus	8.227	< 0.000001*	1.734	−0.172	0.107850
Amygdala	7.513	< 0.000001*	1.584	0.075	0.483763
Lateral ventricles	−4.662	0.000010*	0.983	−0.400	0.000108*
Thalamus	4.097	0.000091*	0.864	0.328	0.001749*
Accumbens	3.870	0.000210*	0.816	0.052	0.624811
Cerebrum	3.422	0.000950*	0.721	0.645	< 0.000001*
Caudate	3.360	0.001161*	0.708	0.393	0.000147*
Globus Pallidus	−1.686	0.095382	0.355	0.286	0.006834
Cerebellum	0.520	0.604284	0.110	0.610	< 0.000001*
Putamen	0.406	0.685326	0.086	0.343	0.001065
MCI vs. AD ADNI					
Hippocampus	−2.826	0.005783	0.596	−0.088	0.396346
Amygdala	−2.409	0.017968	0.508	−0.044	0.674424
Accumbens	−2.155	0.033797	0.454	−0.037	0.724393
Caudate	−1.906	0.059724	0.402	0.144	0.166516
Cerebrum	−1.819	0.072132	0.383	0.408	0.000047
Lateral ventricles	1.303	0.195818	0.275	0.205	0.048072
Globus Pallidus	−1.090	0.278535	0.230	0.109	0.297544
Cerebellum	0.179	0.527974	0.038	0.292	0.004500
Thalamus	0.414	0.679312	0.087	0.137	0.187901
Putamen	−0.365	0.715226	0.077	0.144	0.166516
MCI vs. HC ADNI					
Hippocampus	5.687	< 0.000001*	1.199	−0/080	0.436184
Amygdala	5.698	< 0.000001*	1.201	−0.044	0.674424
Thalamus	4.362	0.000033	0.920	0.272	0.007448*
Lateral ventricles	−4.143	0.000075	0.873	0.073	0.478770
Accumbens	2.936	0.004185	0.619	0.272	0.007448*
Globus Pallidus	−2.876	0.004981	0.606	0.208	0.042244
Caudate	1.917	0.058287	0.404	−0.257	0.011738
Cerebrum	1.540	0.126746	0.325	0.482	< 0.000001*
Cerebellum	0.752	0.453925	0.159	0.428	0.000015*
Putamen	−0.077	0.938544	0.016	0.221	0.030814

Notes: ↑ rows are sorted based on the *p* values for the *t*-test; * $p < 0.0038$ Bonferroni corrected for multiple comparison; *d* represents Cohen's d effect size; MMSE: mini mental state examination.

majority of the analysis was limited to brain areas with more distinct structure, such as the hippocampus. Many semi- and fully-automated segmentation methods have been developed. While these methods have been used more commonly in recent years, the reliability and accuracy of these methods was yet to be fully studied. We used four pipelines of HIPS [10], volBrain [11] CAT [12–13] and BrainSuite [14]. In this study we evaluated their reliability by looking at their ability to discriminate between AD and HC, AD and MCI, and MCI and HC groups with ADNI and MIRIAD databases, whether a correlation existed between them, their correlation with MMSE scores, and comparing their results with past literature. Our results showed strong reliability of HIPS, volBrain and CAT. These methods have been successfully applied to

Table 3
Summary of the independent-sample t-tests comparing volumetric data between different groups of the participants and the correlation of the data with MMSE scores using *BrainSuite* method.

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
AD vs. HC MIRIAD					
Inferior Temporal Gyrus	7.245	< 0.000001*	2.184	0.677	0.000001*
Middle Temporal Gyrus	4.738	0.000029*	1.429	0.697	< 0.000001*
Third Ventricle	−4.354	0.000094*	1.313	−0.522	0.000468*
Superior Temporal Gyrus	3.944	0.000323*	1.189	0.507	0.000722*
Supramarginal Gyrus	3.698	0.000668*	1.115	0.509	0.000683*
Angular Gyrus	3.632	0.000809*	1.095	0.52	0.000498*
Middle Occipital Gyrus	3.543	0.001043	1.068	0.536	0.000303*
Pars Opercularis	2.958	0.005237	0.892	0.45	0.003137
Inferior Occipital Gyrus	2.663	0.011206	0.803	0.383	0.013402
Accumbens	−2.64	0.011866	0.796	−0.481	0.001457
Superior Parietal Gyrus	2.534	0.015392	0.764	0.434	0.004538
Superior Colliculus	−2.532	0.015481	0.763	−0.338	0.030512
Parahippocampal Gyrus	2.447	0.019029	0.738	0.428	0.005294
Cingulate Gyrus	−2.374	0.022629	0.716	−0.356	0.022147
Fusiform Gyrus	2.269	0.028864	0.684	0.155	0.334311
Insula	2.133	0.039251	0.643	0.255	0.107583
Globus Pallidus	−1.995	0.053041	0.602	−0.231	0.146663
Cerebellum	1.979	0.054949	0.597	0.191	0.232289
Basal Forebrain	−1.909	0.063590	0.576	−0.243	0.126172
Anterior Orbito-Frontal Gyrus	1.856	0.070973	0.560	0.234	0.140158
Subcallosal Gyrus	−1.847	0.072330	0.557	−0.207	0.194909
Pars Orbitalis	1.82	0.076458	0.549	0.275	0.081327
Lingual Gyrus	−1.809	0.078196	0.545	−0.316	0.043952
Middle Frontal Gyrus	1.807	0.078496	0.545	0.284	0.071904
Lateral Geniculate Nucleus	1.78	0.082929	0.537	0.253	0.109970
Middle Orbito-Frontal Gyrus	1.744	0.089100	0.526	0.142	0.374241
Temporal Pole	1.591	0.119752	0.480	0.022	0.892295
Post-Central Gyrus	1.501	0.141345	0.453	0.315	0.044533
Hippocampus	−1.241	0.221958	0.374	−0.162	0.311495
Transverse Temporal Gyrus	1.138	0.261864	0.343	0.268	0.090858
Transvers Frontal Gyrus	1.099	0.278660	0.331	0.272	0.085861
Thalamus	0.968	0.339074	0.292	0.087	0.586936
Inferior Colliculus	0.94	0.352768	0.283	0.102	0.526091
Pars Triangularis	0.911	0.367812	0.275	0.216	0.174328
Claustrum	0.87	0.389785	0.262	0.163	0.308227
Caudate	−0.863	0.393478	0.260	−0.122	0.448542
Precentral Gyrus	0.859	0.395408	0.259	0.242	0.128116
Paracentral Lobule	0.858	0.395895	0.259	0.128	0.424284
Superior Occipital Gyrus	0.858	0.395943	0.259	0.169	0.291590
Cuneus	0.843	0.404153	0.254	0.106	0.510235
Putamen	0.653	0.517714	0.197	−0.001	0.995473
Lateral Orbitofrontal Gyrus	−0.548	0.586738	0.165	−0.071	0.658410
Gyrus Rectus	−0.516	0.608533	0.156	−0.123	0.442668
Medial Geniculate Nucleus	−0.505	0.616072	0.152	−0.122	0.448980
Mammillary Body	0.492	0.625193	0.148	0.125	0.436896
Precuneus	−0.49	0.627078	0.148	−0.007	0.967476
Posterior Orbito-Frontal Gyrus	0.445	0.658856	0.134	0.02	0.901794
Brainstem	0.209	0.835439	0.063	−0.015	0.925512
Superior Frontal Gyrus	0.202	0.840699	0.061	0.132	0.411455
Amygdala	0.145	0.885584	0.044	−0.109	0.497327

Table 3 (*continued*)

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
AD vs. HC ADNI					
Lateral geniculate nucleus	4.267	0.000100*	2.184	0.227	0.1237048
White matter (cerebrum)	3.278	0.002018	1.429	0.338	0.0200422
Middle occipital gyrus	3.176	0.002690	1.313	0.201	0.1747845
Thalamus	3.162	0.002799	1.189	0.066	0.6588259
Caudate nucleus	2.861	0.006371	1.115	0.294	0.0444238
Medial geniculate nucleus	2.628	0.011689	1.095	0.204	0.1684918
Angular gyrus	2.270	0.028006	1.068	0.333	0.0217689
Transverse temporal gyrus	2.265	0.028357	0.892	0.031	0.8356742
Parahippocampal gyrus	2.197	0.033209	0.803	−0.176	0.2351574
Precentral gyrus	2.178	0.034677	0.134	0.134	0.3690257
Supramarginal gyrus	2.135	0.038172	0.764	0.071	0.6317679
Superior frontal gyrus	2.085	0.042752	0.763	0.085	0.5671476
Globus Pallidus	2.076	0.043620	0.738	0.256	0.0823122
Pars Opercularis	2.073	0.043920	0.716	0.172	0.2460719
Middle frontal gyrus	2.068	0.044320	0.684	0.204	0.1689554
MCI vs. AD ADNI					
Background	3.365	0.001325	2.184	0.237	0.063059
Supramarginal gyrus	−3.241	0.001929	1.429	0.040	0.754590
Middle occipital gyrus	−2.961	0.004354	1.313	0.110	0.389691
Superior frontal gyrus	−2.755	0.007715	1.189	−0.044	0.726853
Precuneus	−2.614	0.011236	1.115	0.058	0.649361
Paracentral lobule	−2.408	0.019034	1.095	0.095	0.458392
Middle frontal gyrus	−2.284	0.025817	1.068	0.116	0.362751
Pars Opercularis	−2.267	0.026883	0.892	0.072	0.573541
Precentral gyrus	−2.141	0.036273	0.803	0.061	0.632649
Angular gyrus	−2.046	0.044993	0.796	0.286	0.022251
MCI vs. HC ADNI					
Background	4.512	0.000020*	2.184	−0.222	0.063739
Lateral geniculate nucleus	3.447	0.000974*	1.429	0.138	0.254521
Parahippocampal gyrus	3.007	0.003688	1.313	−0.053	0.662537
Thalamus	2.631	0.010510	1.189	0.007	0.953683
White matter (cerebrum)	2.243	0.028133	1.115	−0.245	0.040858
Temporal pole	2.161	0.034184	1.095	0.007	0.947772
Medial geniculate nucleus	2.108	0.038669	1.068	0.105	0.384962

Notes: ↑ rows are sorted based on the *p* values for the *t*-test; * $p < 0.001000$ Bonferroni corrected for multiple comparison; *d* represents Cohen's d effect size; MMSE: mini mental state examination.

brain images from those with AD and MCI [27].

BrainSuite, however, underperformed greatly. For example, it failed to accurately segment the hippocampus, thalamus and amygdala to show a significant difference between the two groups. While this automatic segmentation method has been used frequently in past research [28] its application has been mostly limited to the processing of brains with no atrophy [29], as well as detection of gross segments such as tumours [30]. Given that early AD is so difficult to recognise, being able to detect atrophy represents a crucial aspect to diagnosing AD earlier and consequently providing such subjects with better preventative measures, thus helping to ensure an extended period of higher quality of

Table 4
Summary of the independent-sample t-tests comparing volumetric data between different groups of the participants and the correlation of the data with MMSE scores using *HIPS* method.

	Comparison between groups			Correlation with MMSE	
Brain Area	*t*	*p*↑	*d*	*r*	*p*
AD vs. HC MIRIAD					
SR-SL-SM	8.990	< 0.000001*	2.843	0.393	0.000004*
Hippocampus	8.619	< 0.000001*	2.726	0.388	0.000005*
CA4/Dentate Gyrus	8.248	< 0.000001*	2.608	0.402	0.000003*
CA1	6.308	< 0.000001*	1.995	0.256	0.000389*
Subiculum	5.121	0.000007*	1.619	0.229	0.000873*
CA2-CA3	5.025	0.000009*	1.589	0.288	0.000142*
AD vs. HC ADNI					
SR-SL-SM	11.817	< 0.000001*	2.843	0.509	< 0.000001*
Hippocampus	10.291	< 0.000001*	2.726	0.734	< 0.000001*
CA4/Dentate Gyrus	9.1676	< 0.000001*	2.608	0.608	< 0.000001*
CA1	8.3288	< 0.000001*	1.995	0.676	< 0.000001*
Subiculum	6.1087	< 0.000001*	1.619	0.451	< 0.000001*
CA2-CA3	5.6342	< 0.000001*	1.589	0.628	< 0.000001*
MCI vs. AD ADNI					
SR-SL-SM	−2.954	0.003987*	2.843	0.216	0.037005*
Hippocampus	−2.718	0.007847*	2.726	0.328	0.001294
CA4-DG	−2.394	0.018679	2.608	0.218	0.035353
Subiculum	−2.298	0.023831	1.995	0.119	0.254476
CA1	−2.237	0.02771	1.619	0.268	0.009133*
CA2-CA3	−1.407	0.16264	1.589	0.251	0.014895
MCI vs. HC ADNI					
SR-SL-SM	9.390	< 0.000001*	2.843	0.276	0.006597*
Hippocampus	7.947	< 0.000001*	2.726	0.536	< 0.000001*
CA4/Dentate Gyrus	7.505	< 0.000001*	2.608	0.404	0.000048*
CA1	6.157	<0.000001*	1.995	0.462	0.000002*
CA2-CA3	4.334	0.000035*	1.589	0.431	0.000012*
Subiculum	3.709	0.000352*	1.619	0.210	0.040530

Notes: ↑ rows are sorted based on the *p* values for the *t*-test; * $p < 0.008333$ Bonferroni corrected for multiple comparison; *d* represents Cohen's d effect size; MMSE: mini mental state examination; SR-SL-SM: strata radiatum/lacunosum/moleculare.

life for these individuals. This highlights the importance of validation studies such as ours to gain a greater understanding of the applications and limitations of different methods [31], especially considering the greater accuracy and speed identified with our method.

The volume of the hippocampus is considered as an important biomarker for AD and has been included in recently proposed research diagnostic criteria. It has been shown that the hippocampal atrophy estimated on anatomical T1 weighted MRI can help in classifying the different stages of AD. Confirming past literature, our results showed that the hippocampus volume significantly differed between AD and the HC, MCI and HC.

Histological studies have shown that lesions are not uniformly distributed within the hippocampus. Neuronal loss results in a reduction of the thickness of the layers richer in neuronal bodies, while the loss of synapses results in the reduction of the layers poorer in neuronal bodies and these changes are stage-dependent [32]. Our results, however, failed to differentiate the contribution of these subfields in AD; they all showed significant reduction in size, compared to the control group. This effect could be because our AD group consisted of those with later stages of AD. The contribution of different subfields of the hippocampus is more visible in those with MCI [33].

While the contribution of atrophy in the hippocampus has been widely studied, the role of atrophy in the rest of the brain in AD is less clear [17]. An important contributing factor is that the boundaries of the

Table 5
Correlation of the size of common brain areas reported by the three segmentation methods.

		CAT		volBrain	
	Brain Area	*r*	*p*	*r*	*p*
AD & HC MIRIAD					
BrainSuite	Accumbens	−0.227	0.159813	−0.277	0.078996
	Amygdala	0.240	0.136249	0.029	0.858460
	Caudate	0.169	0.298015	0.090	0.577545
	Globus Pallidus	−0.118	0.469153	0.184	0.249666
	Hippocampus	−0.162	0.318747	−0.275	0.081327
	Putamen	0.328	0.039077	−0.186	0.243349
	Thalamus	0.188	0.245249	0.177	0.268448
volBrain	Accumbens	0.633	0.000007*		
	Amygdala	0.632	0.000007*		
	Caudate	0.470	0.001678*		
	Globus Pallidus	−0.245	0.118543		
	Hippocampus	0.637	0.000006*		
	Putamen	0.020	0.898315		
	Thalamus	0.541	0.000214*		
AD & HC ADNI					
BrainSuite	Accumbens	0.316	0.030547	0.1488	0.318314
	Amygdala	−0.139	0.351315	−0.0584	0.696375
	Caudate	0.321	0.027954	0.2909	0.047270
	Globus Pallidus	0.136	0.363220	−0.1772	0.233327
	Hippocampus	0.056	0.710278	0.0183	0.902928
	Putamen	0.257	0.080614	0.0191	0.898464
	Thalamus	0.135	0.364101	0.1111	0.457192
volBrain	Accumbens	0.766	<0.000001*		
	Amygdala	0.906	<0.000001*		
	Caudate	0.683	<0.000001*		
	Globus Pallidus	0.514	<0.000001*		
	Hippocampus	0.948	<0.000001*		
	Putamen	0.687	<0.000001*		
	Thalamus	0.529	<0.000001*		
AD & MCI ADNI					
BrainSuite	Accumbens	0.0858	0.503728	0.1488	0.318314
	Amygdala	0.1048	0.413672	−0.0584	0.696375
	Caudate	0.2066	0.104172	0.2909	0.047270
	Globus Pallidus	0.0164	0.898526	−0.1772	0.233327
	Hippocampus	0.1232	0.335944	0.0183	0.902928
	Putamen	0.0406	0.752292	0.0191	0.898464
	Thalamus	0.0771	0.548096	0.1111	0.457192
volBrain	Accumbens	0.788	<0.000001*		
	Amygdala	0.887	<0.000001*		
	Caudate	0.838	<0.000001*		
	Globus Pallidus	0.640	<0.000001*		
	Hippocampus	0.935	<0.000001*		
	Putamen	0.837	<0.000001*		
	Thalamus	0.617	<0.000001*		
MCI & HC ADNI					
BrainSuite	Accumbens	0.0343	0.778016	0.149	0.318314
	Amygdala	0.1747	0.148121	−0.058	0.696375
	Caudate	0.1068	0.378955	0.291	0.047270
	Globus Pallidus	−0.0713	0.557286	−0.177	0.233327
	Hippocampus	0.1840	0.127416	0.018	0.902928
	Putamen	0.0034	0.977883	0.019	0.898464
	Thalamus	0.1980	0.100416	0.111	0.457192
volBrain	Accumbens	0.762	<0.000001*		
	Amygdala	0.906	<0.000001*		
	Caudate	0.619	<0.000001*		

(*continued on next page*)

Table 5 (*continued*)

		CAT		volBrain	
	Brain Area	*r*	*p*	*r*	*p*
	Globus Pallidus	0.524	<0.000001*		
	Hippocampus	0.933	<0.000001*		
	Putamen	0.689	<0.000001*		
	Thalamus	0.623	<0.000001*		

Notes: * $p < 0.002380$ Bonferroni corrected for multiple comparison.

hippocampus are easier for human operators or automated algorithms to recognise than other brain areas such as the amygdala, entorhinal cortex or thalamus [17]. Due to methodological advances, however, it is now possible to measure atrophy across the entire cortex with good precision. Our results from CAT and volBrain methods showed strongly significant differences between many brain areas such as the amygdala, thalamus, nucleus accumbens, insula and caudate. These findings are in-line with past literature showing similar differences in these brain areas [17].

There is a growing body of literature showing a correlation between cognitive decline and brain atrophy. For example, it has been shown that basal forebrain changes are correlated with cognitive decline in MCI and AD patients, as measured with recall task and MMSE, as well as healthy participants that later progressed to AD. Atrophy of other brain areas such as lateral and medial parietal cortex, as well as lateral temporal cortex have also been shown to have a correlation with cognitive decline [34]. Our results showed a strong correlation between brain atrophy and cognitive decline as measured by MMSE. All brain areas that were significantly different between the AD and the control group showed a significant correlation with MMSE, except for the caudate (CAT $p = 0.001155$, volBrain $p = 0.005091$, Bonferroni corrected statistic not significant). While the effect of shrinkage of the caudate in AD is not very clear, there is some evidence that caudate volume has a correlation with MMSE measures, although not as strongly as other brain areas such as the thalamus [35]. An important consideration is that atrophy in the left caudate has a stronger role in AD, as compared to the right caudate [36]. Our analysis combined both the left and right caudate, which may have led to this inconsistency between our results and previous literature.

Although AD commonly presents as an amnestic syndrome, there is significant heterogeneity across individuals, which is accompanied by different atrophy patterns [26]. For example, while those with more language difficulties might exhibit greater atrophy in temporal or parietal regions, those with more visual difficulties might have greater atrophy in posterior cortical regions [37]. Availability of the automated systems offers many opportunities, such as the ability to analyse a large number of brain images with reasonable time and expertise. This is in particular very appealing, considering the increased number of large datasets such as MIRIAD and ADNI. Automated systems can go through the collection and aggregate data from a wide range of participants, healthy and patients to gain a greater understanding of AD. Methods with advanced accuracy and speed can analyse such banks with accuracy such that their applicability to clinical settings is inevitable with ongoing technological and practical advancements. This is important considering the heterogeneity of the disease and its progression.

Another application of automated systems is in clinical settings. By the time of diagnosis, rapid ongoing atrophy is already far advanced. Early diagnosis of AD in MCI stage can help with deceleration of the progression of the disease. This is particularly important as there are modifiable factors that can help with brain health. Therefore, a massive effort has been devoted to the development of diagnostic methods to enable researchers and clinicians to detect AD and MCI and cases with potential progression to AD, as early as possible. For the development of preventive strategies, it is important to predict future brain atrophy, as this may aid in identifying which individuals with normal cognition are more susceptible of progressing to later stages of AD [38]. Clinician's reliance on their own expertise and subjective judgements arises from caution held over automated systems due to their lower performance. However, with recent developments and methods, automated systems can provide additional information to clinicians, enabling them to have a greater understanding of the progression of the atrophy [39]. Some of these methods have already received approval from different licensing bodies such as CE (European conformity) and FDA (food and drug administration, USA) approval. These methods, however, come with some limitations such as speed of processing, expensive licences, or requirement of other specialised software. This study is another step to evaluate freely available analytical tools to achieve an ideal analysis pipeline, suitable for researchers and clinicians. Ultimately, such work serves to aid clinicians in their diagnoses of future MCI and thus AD, as well as to help improve the preventative measures taken to help secure a greater quality of life for subjects with AD. Clinicians still rely heavily on subjective judgement, which requires great expertise. Agreeing with the reviewer, clinicians use automated segmentation methods very cautiously due to their poor performance. Therefore, development of methods such as the one suggested in this study can pave the way for further application of automated methods in clinical settings.

Availability of the reliable automated segmentation methods enables researchers and clinicians to have a greater understanding of the underlying mechanisms and the progression of the AD. This will allow them to attempt to prevent or decelerate the progression of the disease more effectively. This rate can be helpful to have a more informed understanding whether an individual with MCI will later progress to AD or not. The output of automated segmentation methods can also be used in training of intelligent classification methods such as those using artificial neural networks and support vector machines, which has shown promising results [40].

The purpose of this article was not to identify the superiority of any particular automatic segmentation method over another, but to solely highlight possible limitations and applications of four commonly used segmentation methods. We proposed that CAT, volBrain and HIPS are methods that can robustly operate on brain images with significant atrophy and can be used in research and clinical settings. BrainSuite, however, should be used with caution for brain images with atrophy.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocn.2022.03.004.

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