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Neuroimaging on Brain Aging and Neurodegeneration

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Neuroimaging in brain aging and neurodegeneration

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by

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Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that usually affects but not limited to the elder population. We see that when an individual develops AD, the brain cells are degenerating and dying at rates that are uncontrollable. Worldwide AD has affected at least 50 million people and we will continue to see this number increase. Although the research done on AD has made great strides, much is still unknown and being studied. Previous studies have allowed us to understand that many of the impacts of AD are correlated to various regions of the brain experiencing atrophy. This causes an individual's cognitive, behavioral, and social skills to be impaired. This paper examined the question of how the use of structural magnetic resonance imaging (MRI) can be used for studying brain atrophy in AD. Using the software, FreeSurfer, we estimated the cortical volumes of various brain regions in AD patients (n = 42) and subjects without AD (n = 38). We found that compared to non-demented individuals, demented patients experience more atrophy in multiple brain regions such as the cerebral cortex gray and white matter of entorhinal, parahippocampal, fusiform, insula, inferior temporal, superior temporal, rostral middle frontal, and lateral occipital gyri, as well as subcortical structures, such as amygdala and hippocampus.. Although the sample used was relatively small, we expect that using structural MRI scans of larger samples will increase the ability to rely on volumetric data to help us further understand what regions are being impacted by AD and provide further support for early diagnosis of AD.

Introduction:

Dementia and its Cause:

In the United States, neurodegenerative diseases affect an estimated 5.3 million people, most patients being over the age of 65. Dementia incidence increases exponentially between ages of 65 and 90 years and doubles approximately every 5 years (Corrada et al. 2010). Multiple pathological processes can lead to neurodegeneration, but the condition itself involves neurons in the brain losing function over time and eventually dying. The brain is made up of billions of neurons; each of them have a unique structure and function, and they form synapses with other neurons. The communication between neurons allow us to do daily things like thinking, moving, and talking. Dementia is a clinical syndrome that often results from a neurodegenerative disease process. It is a condition that causes loss of memory, language, problem-solving, and other thinking abilities. There are millions of individuals living with dementia and it is predicted that by 2050 it will continue to increase to 152 million (Livingston et al. 2020). The most common forms of dementia are AD, Lewy body dementia, and vascular brain injury. The severity of the disease will differ depending on the individual, but it can be as severe as losing all of one's cognitive abilities and ability to function independently in daily life. Doctors and researchers today still do not fully understand all pathological processes that can lead to dementia. Early prevention, diagnosis, and a cure are key focuses in dementia research. Understanding the different type of neurodegenerative diseases, processes that occurs during certain stages of the diseases, and the effect on the brain are critical to finding a cure, supporting early diagnosis, and developing preventative measures.

The most common type of dementia is AD. This is a progressive and irreversible disease that causes the symptoms of dementia to gradually worsen over time. Studies have shown that

this brain disorder typically occurs after the age of 65 (Bature et. al 2017). This disease causes

brain atrophy and loss of synaptic connections between networks of neurons. The pathological

hallmarks of AD contain neuritic plaques, consisting mainly of a pathological protein, amyloid β

(Aβ) and neurofibrillary tangles (NFTs), which are intraneuronal deposits of pathologically

phosphorylated form of microtubule protein tau. Aβ accumulation between nerve cells is toxic to

the surrounding cells, causes inflammation, and disrupts cell function. NFTs form inside neurons

and disrupt axonal trafficking and other cell functions, and cause difficulty with synaptic

communication between neurons.

Even though AD is a neuropathological diagnosis and cannot be made based on clinical

symptoms, there are some correlations between clinical findings and pathology. There are three

different stages of AD: mild, moderate, and severe. During the mild stage of AD, the individual

is able to function normally with some symptoms. Some specific signs of mild AD are

difficulties with coming up with the right word or name, remembering names when introduced to

new people, having difficulty performing tasks in social or work settings, forgetting material that

was just read, losing or misplacing a valuable object, and experiencing increased trouble with

planning or organizing (Stages of Alzheimer's). The symptoms are not very apparent, and special

neuropsychological testing is required for diagnosis of cognitive impairment during this stage.

During the moderate stage of AD, individuals experience greater struggles to perform daily life

compared to the individuals with mild AD. This stage is the usually the longest stage, requires

more care, and the symptoms are more pronounced. Although symptoms vary from individual to

individual, common symptoms that arise during this stage of AD are being forgetful of events or

personal history, feeling moody or withdrawn, being unable to recall information about

themselves like their address or telephone number and the school they attended, experiencing

confusion about where they are or what day it is, requiring help choosing proper clothing for the season or the occasion, having trouble controlling their bladder and bowels, experiencing changes in sleep patterns, showing an increased tendency to wander or become lost, and demonstrating personality and behavioral changes (Stages of Alzheimer's). Lastly, during the severe stage of AD, which is the final stage and is very hard for the patient and their caregivers. Common symptoms during this stage are the need for around-the-clock assistance with daily personal care, losing awareness of recent experiences as well as of their surroundings, experiencing changes in physical abilities like walking, sitting, and swallowing, difficulty with communication, and becoming vulnerable to infections, especially pneumonia (Stages of Alzheimer's). The individual is incapable of almost all tasks, abilities, and skills on their own and there are currently no measures to help them get better. There are very limited to no solutions to help with progression and the symptoms.

Another neurodegenerative disease that causes dementia is Lewy body disease (LBD). This disease is characterized by presence of Lewy bodies, which are intracellular deposits of a pathological form of synaptic protein α-synuclein. α-synuclein deposition causes neuronal dysfunction and eventually death. One type of LBD is Parkinson's disease. In Parkinson's disease, Lewy body formation begins in brainstem, and initially leads to motor impairment, but the process can eventually reach cerebral cortex and cause dementia. Another common type of LBD is Lewy body dementia, a form of dementia that results from the development of Lewy bodies in nerve cells in cerebral cortex. Like AD, LBD has various stages that describe the symptoms of the disease over time. There are seven stages of Lewy Body dementia which are referred as: no cognitive decline, very mild cognitive decline, mild cognitive decline, moderate cognitive decline, moderately severe cognitive decline, severe cognitive decline, and very severe

cognitive decline. Similar to AD as the stages progress, the symptoms get worse and the patients need more assistance and lose more abilities functionally and mentally. Some key clinical features of Lewy body dementia are postural instability, repeated falls, syncope or other transient episodes of unresponsiveness, severe autonomic dysfunction, hallucinations, systematized delusions, apathy, and anxiety and depression (Outeiro et al. 2019). Unlike AD, there is less memory impairment and the difficulty occur more often with visuospatial, attentional, and frontal executive functions (Donaghy and McKeith 2014).

Lastly, vascular brain injury is a result of age-associated pathology in large cerebral arteries as well as smaller vessels which is a potential risk factor for cognitive decline and dementia in older individuals (Scuteri et al. 2011). A type of vascular brain injury is microvascular brain injury (μVBI), and what occurs is that blood flow in large vessels are mostly impaired and a development of pathology in small blood vessels (arterioles). μVBI does not cause large strokes, but histological examination of the brain shows many small lesions that are less than 5mm in the cerebral cortex and subcortical structures. Due to impairment in blood transportation to the brain, it can lead to problems with reasoning, planning, judgement, memory, and other cognitive abilities. People diagnosed with vascular dementia tend to live four around five years after diagnosis, which is less than the average life span for patients with AD (Strand et al. 2018). Both vascular brain injury and LBD are known causes of dementia, but AD is the main focus of study since it has and still affects so many individuals.

Alzheimer's Disease

For many years, much research has been done hoping to a cure for Alzheimer's disease (AD) and related dementias. Although the cure of AD has not yet been discovered, the

advancement of modern medicine and research have accelerated ways to combat symptoms for a short period of time. AD symptoms vary among patients, but symptoms continue to worsen over time. Currently, there are two U.S. Food and Drug Administration (FDA) approved medications that treat the cognitive symptoms of AD: cholinesterase inhibitors and memantine, which is a NDMA receptor antagonist (Medications for Memory). These medications are used to treat symptoms such as memory loss, confusion, and problems with thinking and reasoning. When focusing on the effects of medications to help with the disease, it is critical to understand how the brain is changing volumetrically and pathologically. The brain will decrease volumetrically when diagnosed with this disease because the pathology causes the death and decrease of neurons. Cerebral atrophy has a strong correlation with cognitive decline (Johnson et al. 2012).

The imaging technique called magnetic resonance imaging (MRI) provides detailed structural images of the brain that help us assess the volume its regions. Using the volumetric data from the MRI, scientists are able to examine which regions of the brain experience atrophy during the progression of AD. Structural MRIs provides a technique to understand the disease in living and non-living individuals. MRIs are uncommonly performed on post-mortem brains because MRIs are costly, we do not see this type of imaging done close to death. But the information it provides will be particularly important for understanding the stages of AD by providing correlation with autopsy finding. Autopsies are the only technique to confirm the diagnoses of the disease.

Techniques to diagnose possible AD during life:

If AD is suspected, information on an individual's health changes are inquired, which includes types of medication the individual is taking, their diet, alcohol use, any previous

medical health issues, and any psychological changes. When evaluating the psychological

changes, they want to see if there are any recognizable changes in their behavior, personality,

and cognitive ability. To test their cognitive ability, a Mini-Mental State Exam (MMSE) can be

given to test orientation, attention, memory, language, and visual spatial skills. The MMSE is a

short screening tool that provides an overall measure of cognitive impairment in clinical,

research, and community settings (Arevalo-Rodriguez et al. 2015). The highest score that can be

achieved is a 30, a score 24-20 indicates mild dementia, 20-13 is considered moderated

dementia, and 13 and below suggests severe dementia. Another test often used is the Mini-Cog

test, which is a brief cognitive screening test that consist of two components, a delayed three-

word recall and the clock drawing test (Borson et. al 2005). This is not a diagnostic test for AD

or any other forms of dementia but assesses cognition. A total score under 5 indicates the

likelihood of dementia. Additionally to the cognitive tests, laboratory tests such as blood count,

blood glucose, urinalysis, cerebrospinal fluid, and analysis of the thyroid function can help

exclude other potential causes of dementia and indirectly confirm the diagnosis of suspected AD.

Next, a neurological exam is used to rule out any other conditions similar to AD. This exam tests

the patient's reflexes, coordination, muscle tone and strength, eye movement, speech, and

sensation. Lastly, brain imaging is another method to diagnose brain changes suggestive of AD.

Different brain imaging techniques that can help with diagnosis are computed tomography (CT),

structural and functional MRI, and positron emission tomography (PET). These different types of

brain scans allow doctors to find any identifiable characteristics of AD, like amyloid plaques,

and identify other medical problems like strokes or tumors, which are also cause of dementia.

  There are two common types of MRI, one provides detailed images of organs and tissues

in the brain (structural MRI) and the other measures the brain activity by looking at blood flow

in the brain (functional MRI). Structural MRIs provide the shape and size of cortical regions, which can provide information about the cumulative neuronal damage which is in turn responsible for the clinical state. Previous research has shown that structural MRI is an effective noninvasive technique that provides excellent spatial resolution and good contrast. The images provide information about the size of brain areas and brain atrophy and therefore, the extent of the neurodegeneration can be identified (Lama et al. 2017). MRI scans are expensive and are usually performed if there is a specific reason for doing so like a fall or suspected tumor or stroke. Although MRI is expensive it could be very useful to monitor the progression of the disease by revisiting the volume of the affected regions, to allow for comparisons with previous images.

MRI could provide some insight in the early stages of AD. Early diagnosis is very important because it allows patients to make lifestyle changes that slow down the disease. There are many medical conditions and lifestyle factors that increase the chance of cognitive impairment and AD. Possible ways to prevent or delay the development of AD are controlling hypertension, smoking, obesity, and diabetes. Additionally, mental health disorders like depression and infrequent participation in stimulating activities are factors that increase the chance of mild cognitive impairment (MCI) and AD (Rasmussen and Langerman 2019). Many individuals in the world experience these factors increasing the chance of AD, so it is especially important for doctors to pay attention to warning signs of AD. Previous finding based on imaging data has shown that there is a long preclinical and presymptomatic period where the pathological effects of AD are detectable (Johnson et al. 2012). Using MRI for examining certain brain regions is extremely helpful in understanding the disease, as brain regions showing most

significant rates of atrophy alter as the disease advances, and regional atrophy is already

occurring long before the onset of symptoms (Scahill et al 2002).

Brain Regions and their Functions

In this study, the parts of the brain that were being evaluated were the cerebral cortex

gray and white matter of entorhinal, parahippocampal, fusiform, insula, inferior temporal,

superior temporal, rostral middle frontal, and lateral occipital gyri, as well as subcortical

structures, such as amygdala and hippocampus. The gray and white matter are the tissues that

make up the central nervous system. These are where the cell bodies, dendrites, axon terminals,

synapses, axons, and myelin are located. The gray matter contains cell bodies, dendrites and

axon terminal, which is a major component of the central nervous system. White matter is

composed of nerve fibers that connect neurons to other neurons in different brain regions (Fields

2011).

There are four lobes in the human brain: frontal lobe, temporal lobe, parietal lobe, and

occipital lobe. The regions that were examined were part of three of the major lobes in the brain.

The frontal lobe is the largest of all four lobes; it is important for voluntary movement,

expressive language, and for managing higher level executive functions. In the frontal lobe, you

can find the premotor cortex, primary motor cortex, and Broca's area, which is responsible for

speech production. The temporal lobe is part of the brain near the brain stem and the cerebellum.

It is responsible for emotions, smelling, tasting, perception, memory, and understanding

language. This lobe contains the amygdala, hippocampus, hippocampal regions, and Wernicke's

area, which is responsible for speech comprehension. The function of the amygdala allows

individuals to feel certain emotions, like fear. Amygdala atrophy in AD was prominent in later

stages and is related to the severity of cognitive impairment (Poulin et al. 2011). The parietal lobe is responsible for sensation and perception and the integration and interpretation of sensory information. So, functions such as pain interpretation, visual perception, spatial orientation, and speech are located here. Lastly, the occipital lobe is known as the visual processing center. The function of the lobe is to control vision and its aspects like distance and depth perception, color determination, and facial recognition. The occipital lobe contains the primary visual cortex, which is responsible for processing visual stimuli and assessing the intensity, shape, size and location of objects in the visual fields (Cechetto and Topolovec 2002). In this study we focused on regions which has been shown or are suspected to undergo atrophy in dementia patients at different stage of AD.

Ex vivo imaging of the Brain

Ex-vivo imaging is an uncommon technique, in which the brain is imaged post-mortem, and can be very helpful for studying AD and other neurodegenerative diseases. The information it provides is important because it can help correlate MRI findings with diagnoses. MRI picture of the brain from a person suffering from AD dementia looks very different from a not demented brain (Figure 1). The diseased brain has lost a substantial amount of volume in several regions, including the hippocampus, amygdala, and regions in the temporal lobes. These regions are important for learning and memory, experiencing emotion, and encoding memory, and their loss can explain the loss of corresponding abilities in thinking, movement, learning, memory, and more.
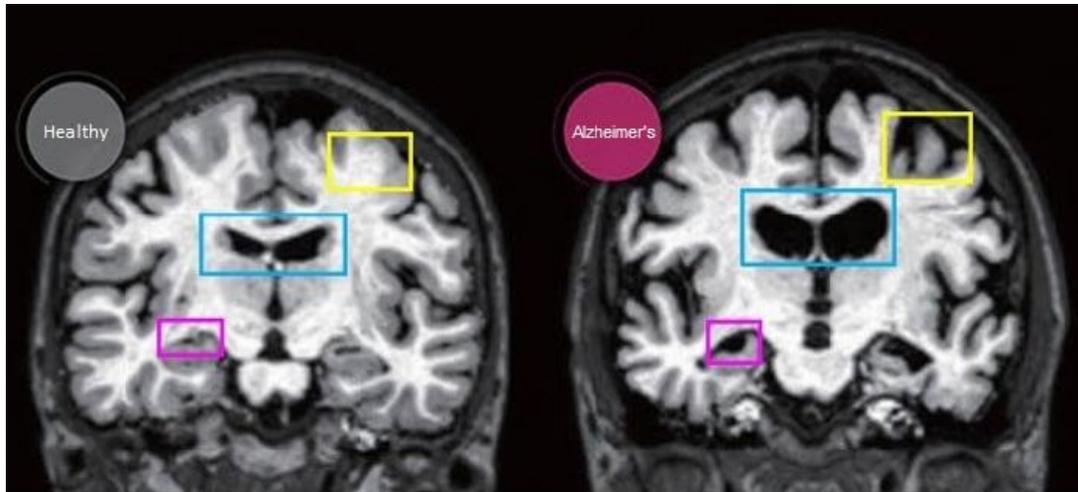
Figure 1. Comparison between a healthy brain and a brain of a n individual with AD. MRI of healthy brain (left) and MRI of Alzheimer's brain (right). Yellow square is displaying the cortex, blue square is displaying the ventricles, and purple square is displaying the hippocampus. (Copyright 2017 by IEEE Institute of Electrical and Electronics Engineers).

Like mentioned before, when an individual is diagnosed with AD, their brain will usually contain Aβ -rich neuritic plaques and NFTs, which is accompanied by neuronal death and loss of synapses (Kocachan and Dogan 2017). Aβ plaques usually appear in the neocortex in the beginning and we will see it present in other regions of the brain as the disease progresses. In order to track the AD neuropathologic change, there is a ranking system that uses three parameters: Aβ plaque score, NFT stage, and neuritic plaque score (Hyman et al. 2012) Aβ plaque score is given by using Thal scoring system, this system has five phases which describes the progressive deposition of Aβ. Phase 1 represents Aβ located in the neocortex, phase 2 shows amyloid in the allocortex or limbic areas, phase 3 is amyloid in the basal ganglia, phase 4 means amyloid is located in the brainstem or midbrain, and phase 5 represents amyloid in the cerebellum. Braak stages are used to describe the degree and positioning of NFTs in the brain.

There are six stages Braak stages I/II represent NFTs located in the entorhinal cortex and regions near it, stages III/IIV represent NFTs in the hippocampus and amygdala, and stages V/VI represent NFTs distributed around the neocortex, affecting primary motor and sensory areas (Braak and Braak, 1991). Another major component in AD pathology are neuritic plaques, which are extracellular deposits of aβ peptides. To characterize aβ plaques and rank the density of them, there is a scoring system developed by Consortium to Establish a Registry for Alzheimer's Disease (CERAD score) (Mirra, 1991). These three scoring systems are known as the "ABC score" and are used to establish a definitive diagnosis of AD. Because this information can only be obtained in post-mortem brains, AD cannot be diagnosed with certainty in living people.

In this study, post-mortem brains were used to examine the structural changes associated with AD using ex-vivo MRI. The goal was to find correlations between ex-vivo MRI data and neuropathological findings in later stage of AD. As we know, brain atrophy is a strong indicator of dementia. Therefore, we hypothesize that demented patients would have greater atrophy compared to non-demented patients.

Materials and Methods:

Human subjects

This study included 80 participants from individuals who were a part of the Adult in Changes (ACT) cohort or University of Washington Alzheimer's Disease Center (ADRC) cohort. The study was approved by the University of Washington Human Subjects Committee. A total of 80 patients participated in this study (Table 1). 42 patients (52.5%) were diagnosed with dementia and 38 patients (47.4%) were diagnosed as not demented. All subjects were

diagnostically evaluated by a board-certified neuropathologist. The diagnostic evaluation included assignments of an "ABC" score and designation of AD neuropathological change, which followed the NIA-AA guidelines (Hyman et al. 2012). The subjects' age, sex, region of examination, and diagnosis were all recorded (Table 1).

Table 1. The demographics, region examined, and diagnosis of the patient of subject (n = 80). Individuals that are "not demented" and are diagnosed with the one of the three diseases occurs because having the disease does not necessarily cause dementia. Dementia is a clinical syndrome, while AD, LBD and VBI are neuropathological diagnoses. Individuals with "no diagnosis" were diagnosed with having any neuropathological disease.

	Demented Patients (n=42)	Not Demented Patients (n=38)
Age (yrs.)	88.5 ± 9.7	90.3 ± 6.3
% of Males	33.33%	42.11%
Right	22	18
Left	17	11
Whole	3	9
% with Alzheimer's Disease	40.4%	25.6%
% with Vascular Brain Injury	4.8%	2.6%
% with Lewy Body	50.0%	25.6%
% with No Diagnosis	2.4%	38.5%

Neuroimaging: Structural MRI

MRI scans were obtained from formalin-fixed brains by using an individual's left, right, or both sides of the brain hemisphere. The right or left hemisphere was chosen at random, unless one hemisphere had visible lesions or hemorrhages. MRI's were taken with 7T Siemens Magnetom MRI scanner using custom-built head coil. Then, the specimens were scanned on a 3T Siemens Tim Trio with a 32-channel head coil. The images were manipulated by using a program called FreeSurfer, it is a software that allows us to produce computerized models of the brains using MRI data. FreeSurfer, when used for the analysis of structural MRIs, it has the ability to provide gray-white matter segmentation, reconstruct the cortical surface, and label regions on the cortical and subcortical structures (Figure 2A). In this study, FreeSurfer provided us with measurements of regional volume. The regions that were analyzed were the cerebral cortex, including both gray and white matter in the entorhinal, parahippocampal, fusiform, inferior temporal, middle temporal, superior temporal, caudal middle frontal, rostral middle frontal, and lateral occipital gyri, insula, hippocampus, and amygdala. (Figure 2B).

A
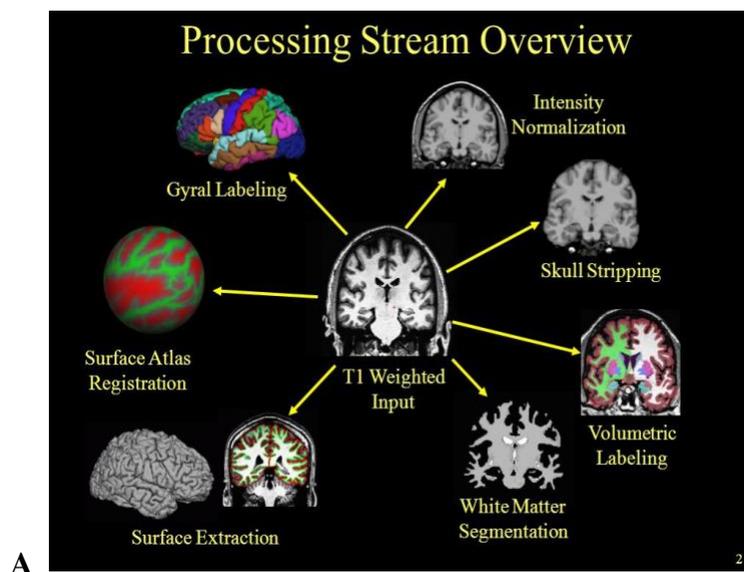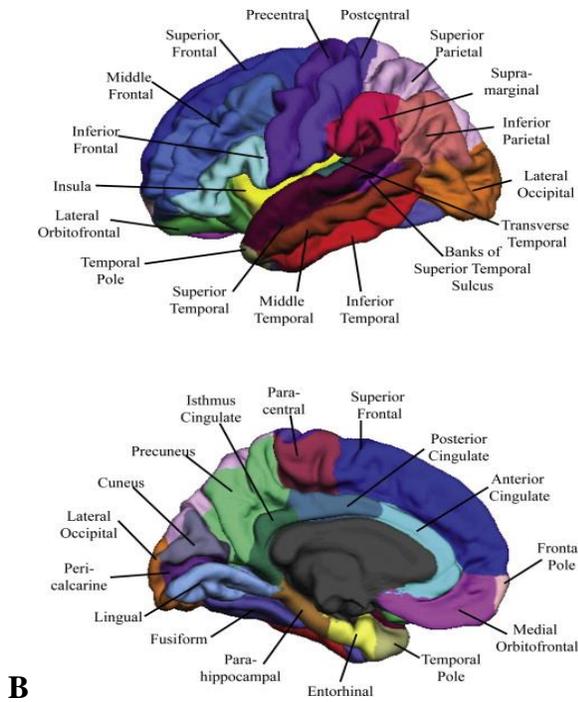
B

Figure 2. Overview of FreeSurfer software and its functions such as volumetric labelling, surface extraction, intensity normalization, gyral labeling, surface atlas registration, white matter segmentation, and skull stripping [A]. Free Surfer's ability to label the different regions in the brain and regions that were used in our experiment [B].

Statistical Analysis: Mann-Whitney U Test

The statistical analyses were performed with Social Science Statistics. Utilizing a Mann-Whitney U test because the data was not normally distributed. This statistical test was used to compare the effects of this neurodegenerative disease on regional volumes. If the sample we were examining was from the left hemisphere, then all the volumes used to normalize were from the left hemisphere and the same was done for the right hemisphere. If it was a whole brain sample, then we would add the volume from the right and the volume from the left and divide it by the sum of the right hemisphere and left hemisphere cerebral cortex and white matter, then divide that all by 2.

Results:

Atrophy in gray and white matter of brain hemispheres

We first sought to quantitatively examine the atrophy in the brain and its regions in demented and non-demented group. We used 22 right-side, 17 left-side, and 3 whole brains for demented patients and 18 right-side, 11 left-side, and 9 whole brains for not demented patients.

To understand the relationship between dementia and its effects on atrophy, we first looked at the volume of the cerebral cortex and subcortical white matter. The mean volume of cerebral cortex per hemisphere for demented patients was $193.4\pm 4.7\ cm^3$ and $219.2\pm 3.3\ cm^3$ for not demented patients (Figure 3A). To see if there was a normal distribution amongst the data, we used a Kolmogorov-Smirnov test. The data showed that it did not follow normal distribution (P = 0.68576). Volume in the cerebral cortex was significantly less in demented patients compared to not demented patients (Mann Whitney-U, P = <0.00001). Then, we looked at the sum of the cortex and subcortical white matter. The average value for demented was $366.3\pm 7.1\ cm^3$ and $411.2 \pm 6.5 cm^3$ for not demented (Figure 3B). The volume in demented patients was significantly less compared to not demented patients (Mann Whitney-U, P = <0.00001)
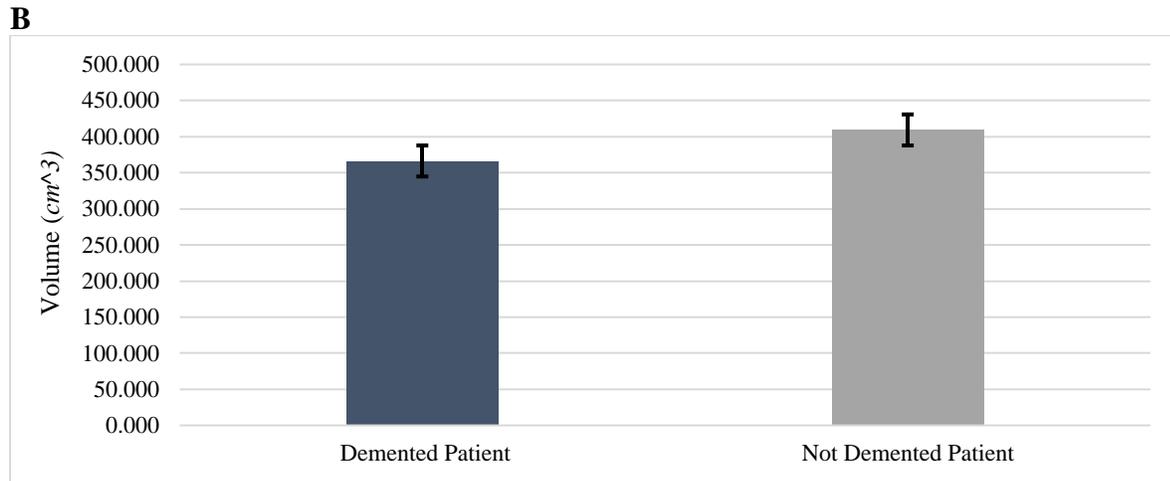
A

B
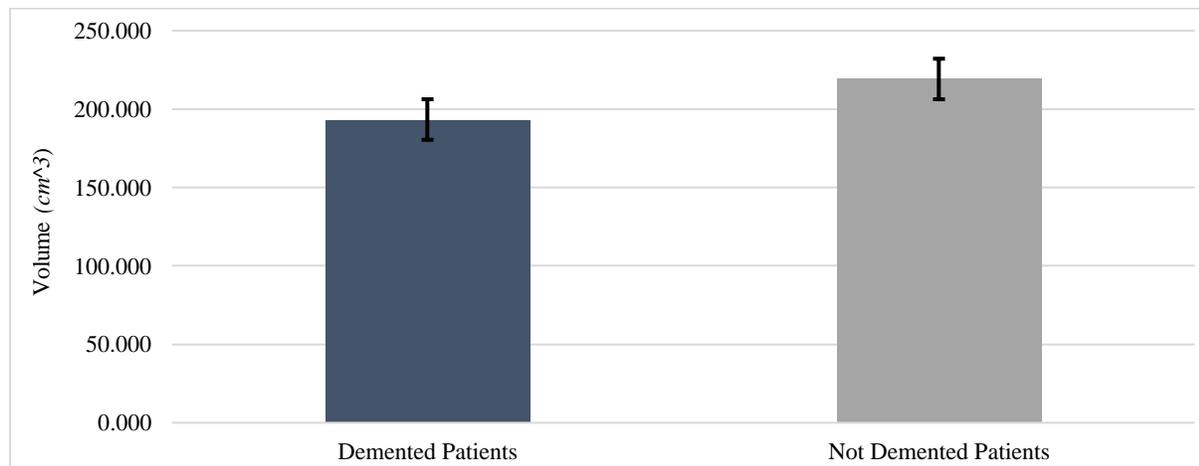
Figure 3. The average cerebral cortex volume [A] and the average sum of cerebral cortex plus white matter [B] between demented (blue) and not demented (grey) patients (n=80).
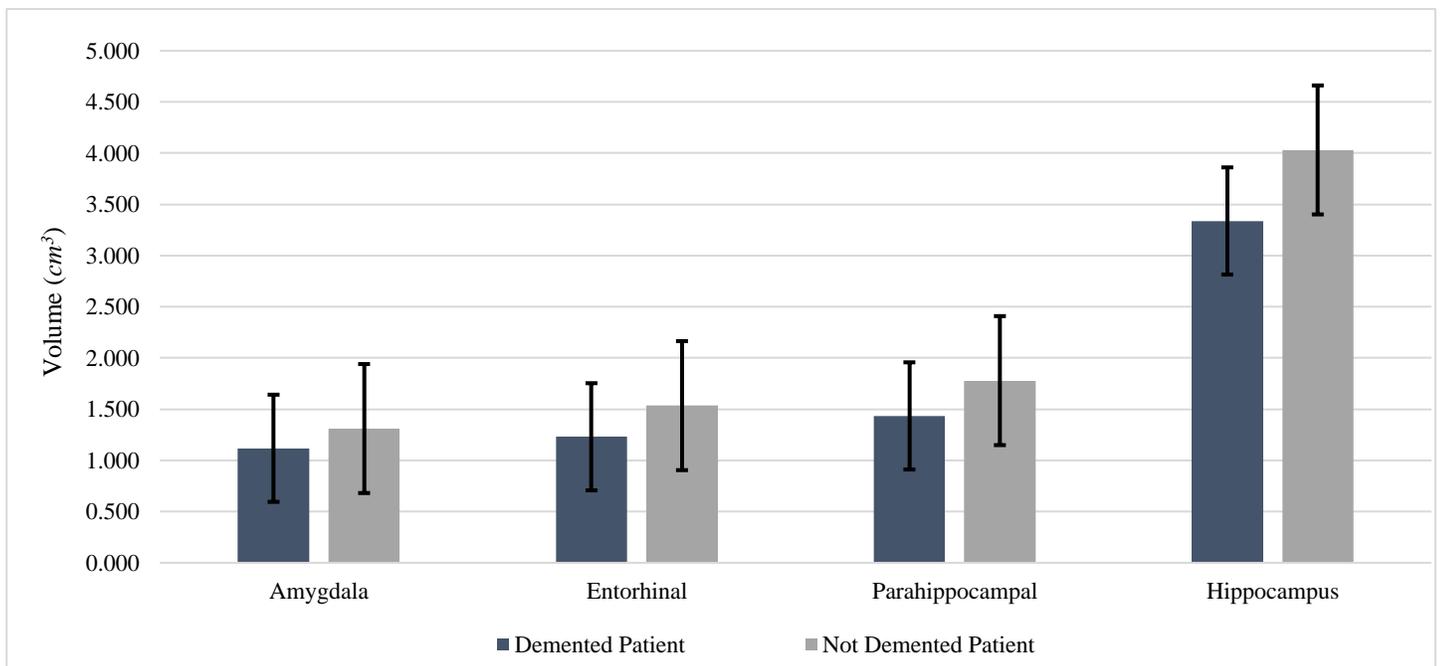
Atrophy in regions in the limbic system

The limbic system is responsible for emotions and memory, therefore we examined volumes of regions in the limbic system like the amygdala, entorhinal cortex, hippocampus, and parahippocampal gyrus between demented and non-demented patients. (Figure 4A). Although the volumes in the amygdala appeared to be different, there was not a statistical difference between the two groups (P = 0.18352). The other regions in the limbic system were significantly different: P = 0.00128 in the entorhinal cortex, P = 0.03236 in the parahippocampal gyrus, and P = 0.00188 in the hippocampus.

Atrophy in other regions of frontal, temporal and occipital lobe

We examined other regions, such as insula, fusiform, inferior temporal, middle temporal, superior temporal, caudal middle frontal, rostral middle frontal gyri, and lateral occipital cortex

(Figure 4B). The average volume for the fusiform gyrus was 6.476 and 8.210, the insula was

6.321 and 7.173, the inferior temporal was 7.958 and 9.830, the middle temporal was 8.474 and

9.988, the superior temporal was 9.776 and 11.561, the caudal middle frontal was 5.141 and

5.442, and the lateral occipital was 10.216 and 11.520. The volumes of caudal middle frontal

gyrus showed no significant difference between the two groups (P = 0.18352). All other regions

showed a significant difference: P = < 0.0001 in the fusiform gyrus, P = 0.00094 in the insula, P

= 0.0001 in the inferior temporal, P = 0.0002 in the middle temporal, P = 0.003 in the superior

temporal, P = 0.00188 in the rostral middle frontal, and P = 0.00126 in the lateral occipital gyri.
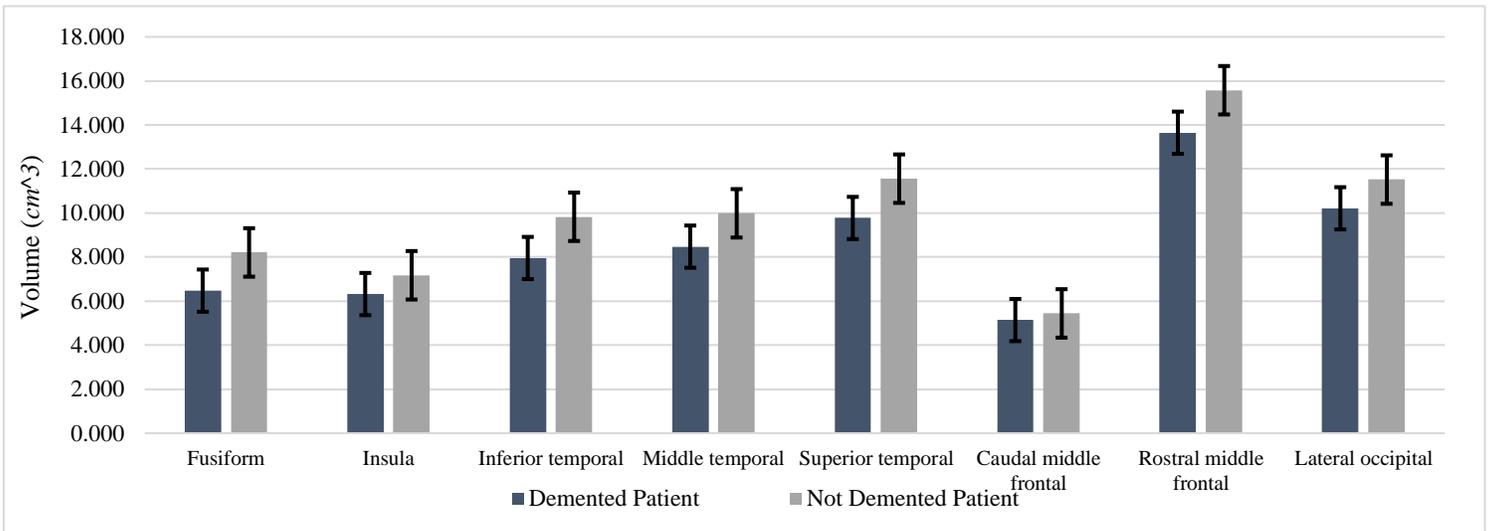
A

B

Figure 4. Volumes of regions in the limbic system and four lobes. Comparisons between regional volumes between demented (blue) and not demented (grey) patients (n=80).

The difference in volume between left and right hemisphere

Of the 80 participants, data from both hemispheres was only available for 12 participants. The rest of the MRI data came from one hemisphere of the brain: 28 from the left hemisphere and 40 from the right hemisphere. Regardless of which hemisphere was being examined, we still wanted to see the relationship between atrophy and dementia. First, we wanted to compare the overall size of the right hemisphere versus the left hemisphere (Figure 5). The left hemisphere's average volume was $210.15 \pm 4.0 cm^3$ and the right hemisphere's average volume was $203.33 \pm 4.8 cm^3$. The left hemisphere appeared larger but was not significantly larger than the right hemisphere (P = 0.15272).
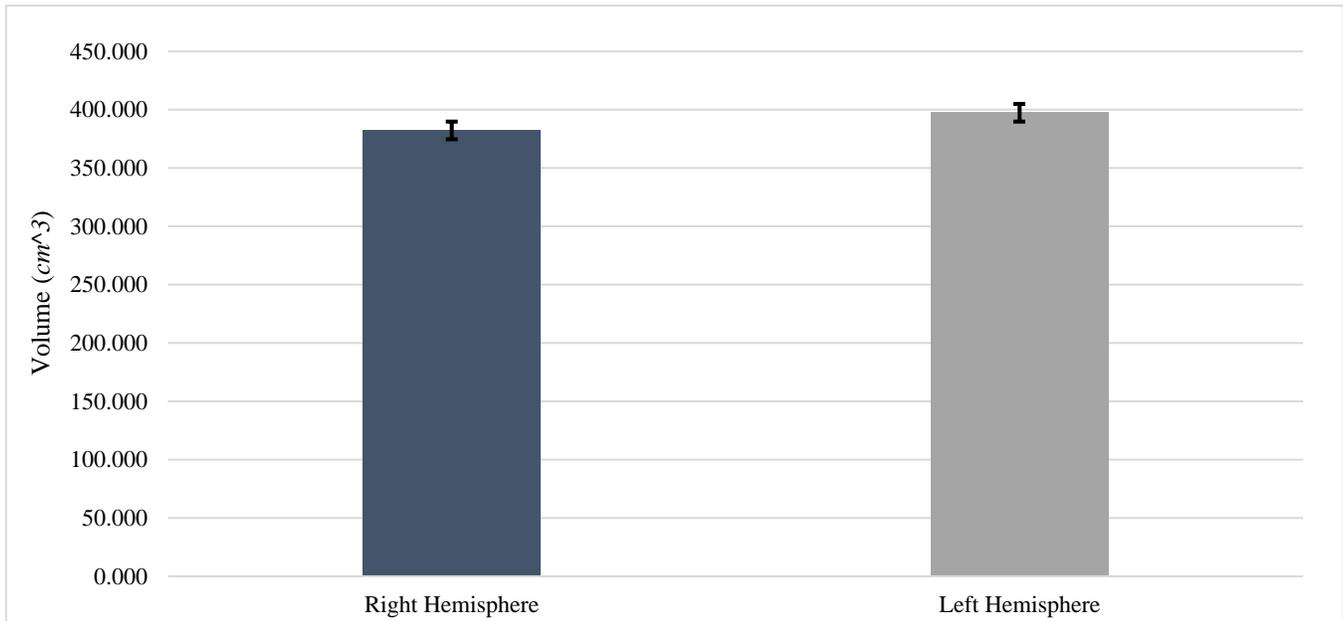
Figure 5. The average sum of the cerebral cortex and white matter volume between the right

hemisphere (blue) and left (grey) hemisphere (n=80).

Next, we looked at the hemispheres separately. Both the right and left hemisphere

samples showed a significant difference between demented and not demented patients (P =

0.0394). The right hemisphere had an average volume of $215.7 \pm 4.8\ cm^3$ for not demented and

$209.1 \pm 4.0\ cm^3$ for demented with a p-value of 0.04 (Figure 6A). The volume of the left

hemisphere was $219.243\ cm^3$ for not demented and $202.796\ cm^3$ for demented patients with a p-

value that is less than 0.0001 (Figure 6B). Overall, we saw that there was a significant difference

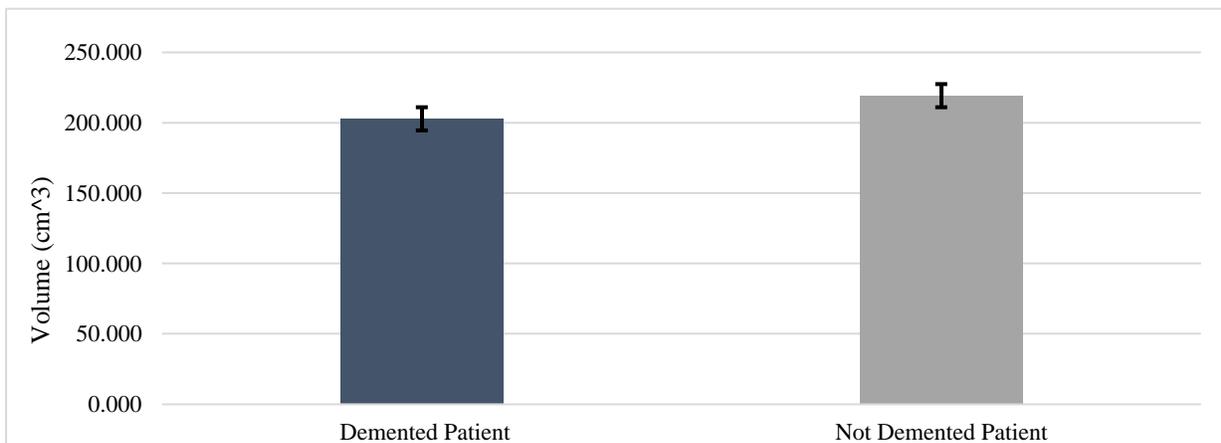between demented patients and not demented patients in both hemispheres.

A
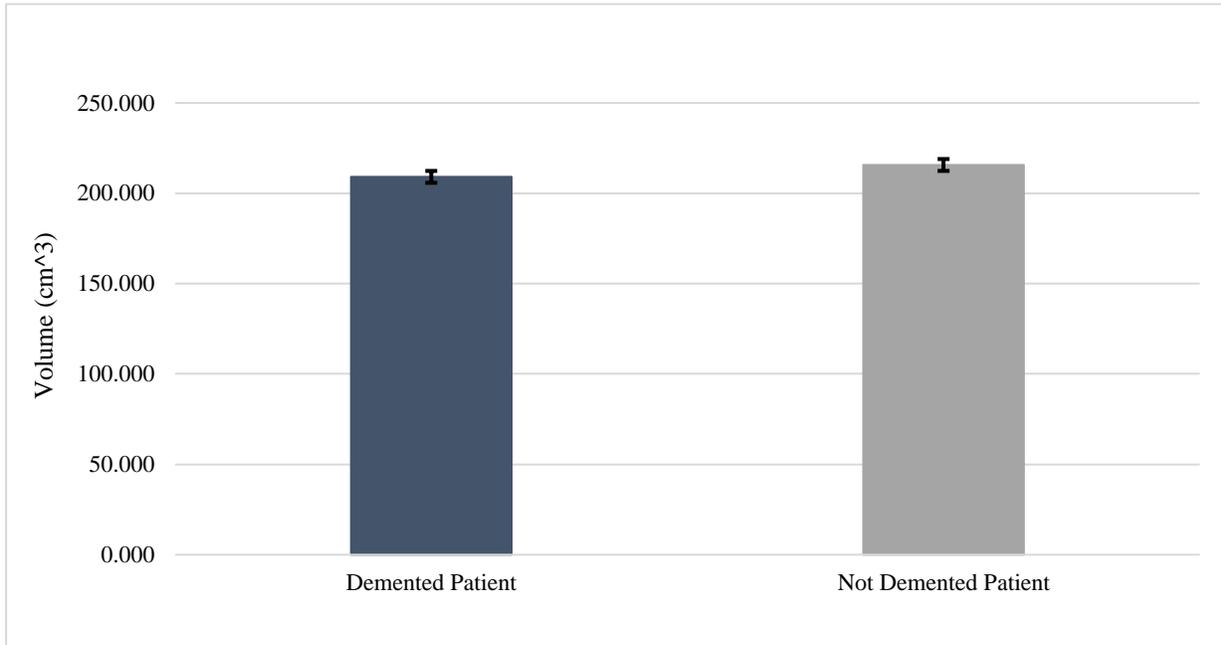
B

Figure 6. The average volume of right hemisphere including the right hemisphere-only of whole brain samples between demented (blue) and not demented (grey) patients (n=50) [A]. The average volume of sum of left hemisphere and left sample of whole hemisphere between demented (blue) and not demented (grey) patients (n=38) [B]

Regions in limbic systems and regions in other lobes in both hemisphere

As shown in Figure 8A, the average volume for the amygdala was 1.235 and 1.368, the entorhinal cortex was 1.445 and 1.439, the parahippocampal was 1.999 and 1.869 and the hippocampus was 3.977 and 4.005 in the right-hemisphere brains. The volumes of limbic regions in the right hemisphere were significantly different: $P = 0.00758$ in the amygdala, $P = 0.03486$ in the entorhinal cortex, $P = 0.03156$ int the parahippocampal gyri, and $P = 0.0038$ in the hippocampus. The average volume for the fusiform was 5.967 and 7.952, the insula was 5.983 and 7.092, the inferior temporal was 7.123 and 9.420, the middle temporal was 8.315 and 10.504, the superior temporal was 9.147 and 11.188, the caudal middle frontal was 4.992 and 5.413, the rostral middle frontal was 13.644 and 16.015, and the lateral occipital was 9.864 and 11.637. (**the 1st t value for averages is demented volume and the 2nd value is not demented volume*). Patients' caudal middle frontal region showed no significant difference when stratified by dementia diagnosis ($P = 0.12114$). In all other regions there was statistical difference: $P = 0.00094$ in the insula, $P = <0.00001$ in the fusiform, $P = 0.00094$ in the middle temporal, $P = 0.0005$ in the superior temporal, $P = 0.00262$ in the rostral middle frontal, and $P = 0.00168$ in the lateral occipital gyrus.

Lastly, in the left hemisphere most comparisons did not yield statistically significant differences. In the regions of the limbic system, we saw a similar trend between volumes, but they were not significant (Figure 10). In the left hemisphere, average volume for the amygdala was 1.196 and 1.289, the entorhinal was 1.417 and 1.582, the parahippocampal was 1.701 and 1.852 and the hippocampus was 3.640 and 3.978 (4B). The p-values for those regions were: $P = 0.28914$ in amygdala, $P = 0.39824$ in entorhinal cortex, $P = 0.2672$ in the parahippocampal gyrus, and $P = 0.28914$ in the hippocampus. The average volume for the fusiform was 7.495 and

8.422, the insula was 6.841 and 7.150, the inferior temporal was 8.916 and 10.238, the middle

temporal was 8.865 and 10.085, the superior temporal was 10.888 and 12.171,the caudal middle

frontal was 5.605 and 5.582, the rostral middle frontal 14.269 and 15.471,and the lateral occipital

was 10.979 and 11.461. The regions that showed statistical differences were: P = 0.02202 in the

fusiform, P = 0.00988 in the inferior temporal gyrus, P = 0.03236 in the middle temporal gyrus,

and P = 0.0601 in the superior temporal gyrus. The insula had a p-value of 0.34722), P = 0.89656

in the caudal middle frontal gyrus, P = 0.14706 in the rostral middle frontal gyrus, (P= 0.14706),

and P = 0.25848) in the lateral occipital gyrus. The regions showed no significant difference

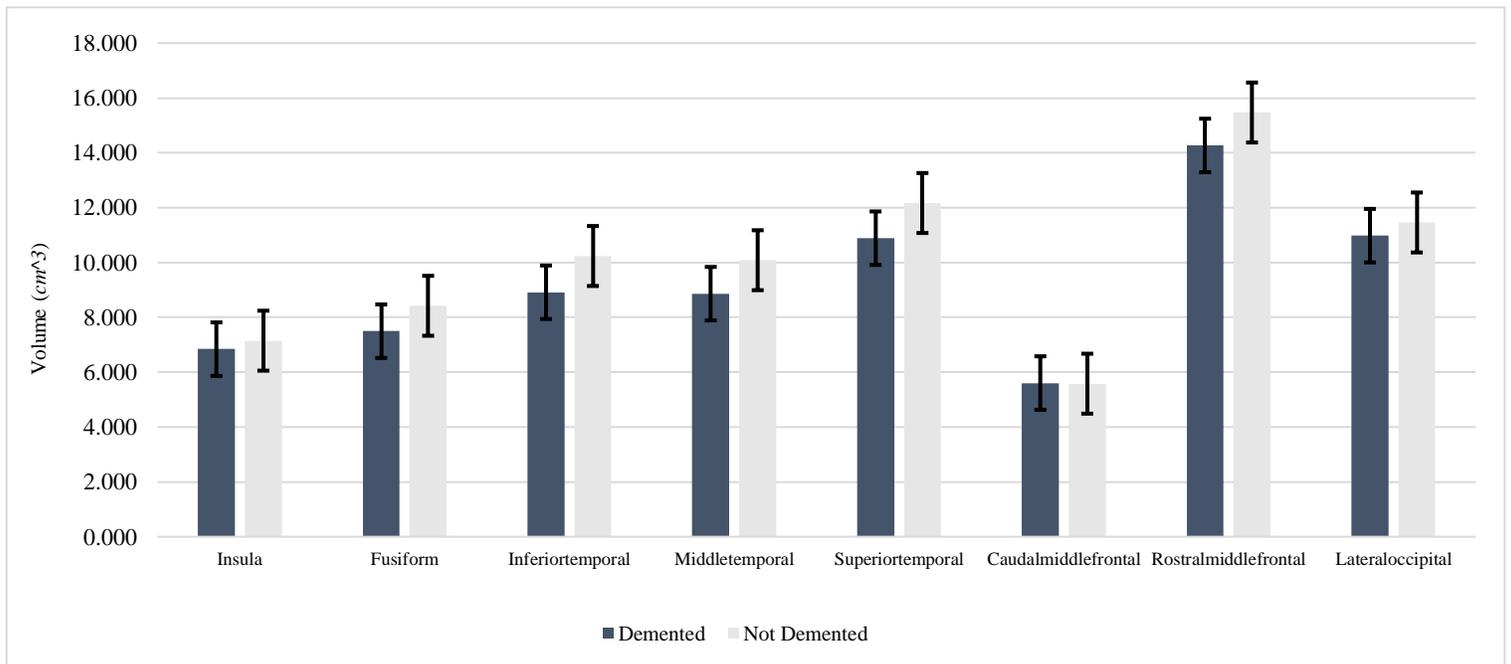between demented and not demented patients in the left hemisphere.
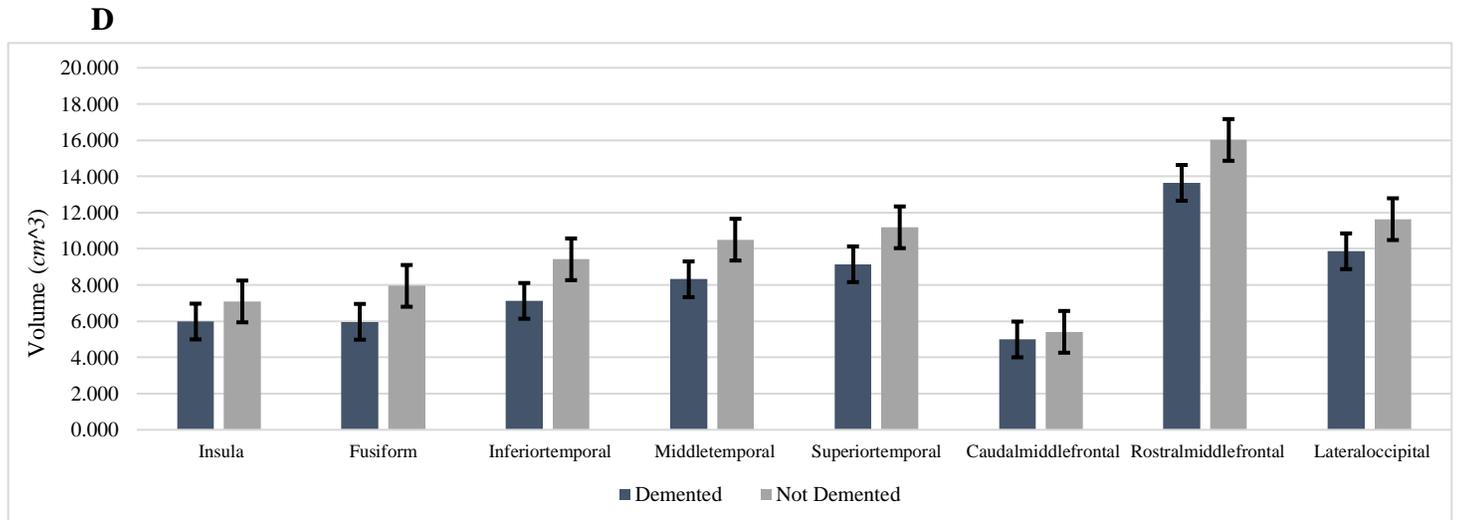
A

B

C

D
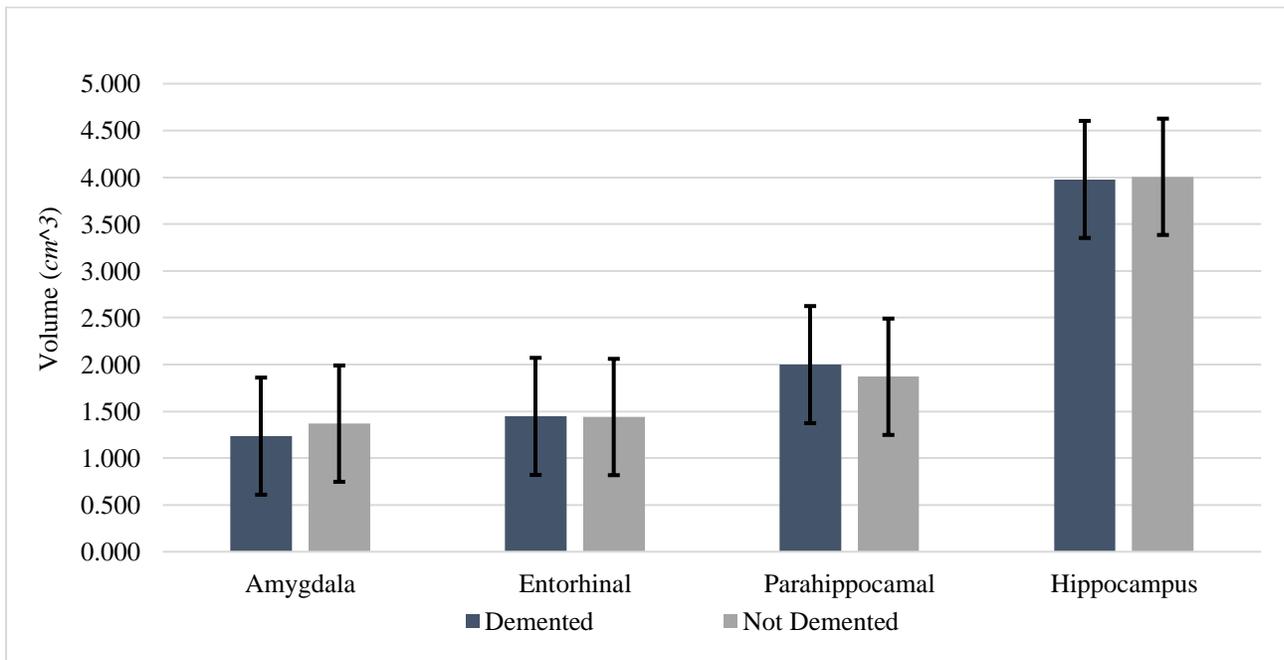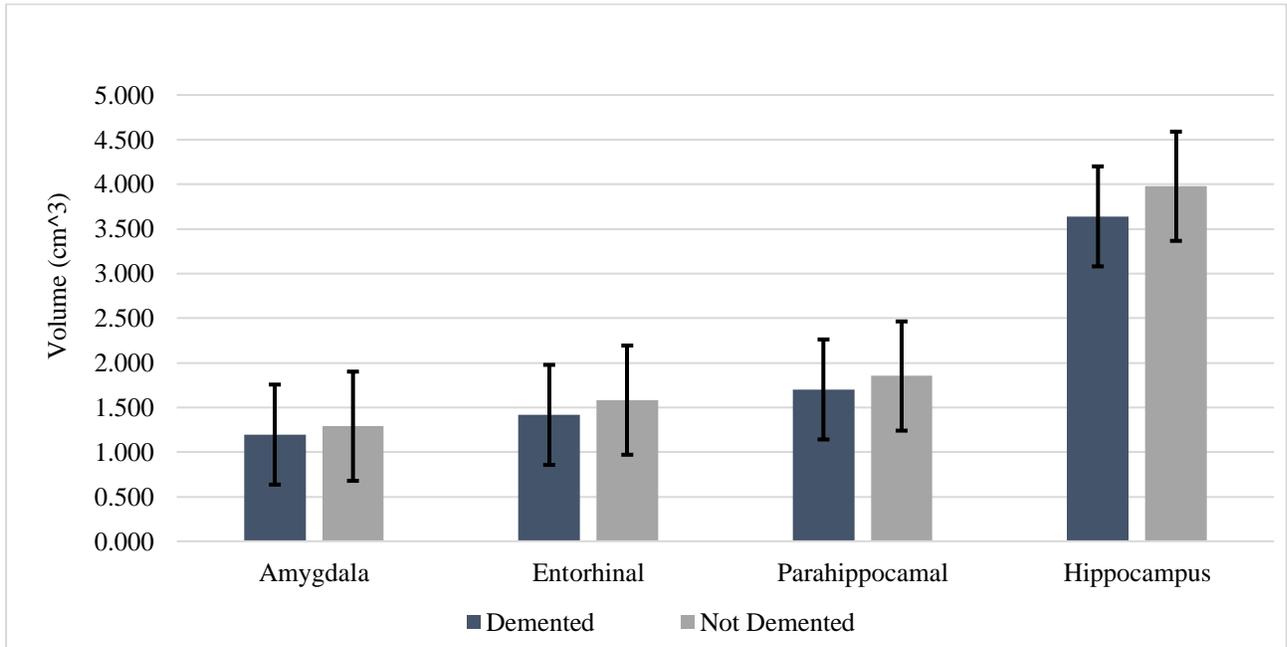
Figure 7. The volumes of regions in the limbic system in the right hemisphere between demented (blue) and not demented (grey) patients (n=50).

Discussion:

AD is continually being studied with the goal of finding ways for early diagnosis, preventative measures, and a cure for this and progressive disease, that is currently irreversible. AD has already affected so many people and will continue to affect millions more. Modern science and medicine have made advances in mitigating the symptoms of AD in early stages, but the search for a cure continues. The aim of this study was to use structural MRIs in post-mortem brains to determine if demented patients experience more atrophy in regions in the frontal, temporal, parietal, and occipital lobes compared to not demented patients, which would be consistent with findings from previous in vivo brain imaging data.

Atrophy in the brain and its lobes

Our results showed that both gray and white matter showed significantly more atrophy in demented patients compared to the non-demented patients. Atrophy in these two regions are

indictors that there is a loss of neuronal bodies as well as processes, resulting in loss of synaptic communication.

Both hemispheres are roughly similar in size, but in most individuals the left hemisphere is slightly larger than the right hemisphere. Our findings showed that when comparing left hemisphere post-mortem brains samples to right hemisphere post-mortem brain samples the left hemisphere tended to be larger, but not significantly in all patients.

The temporal lobe is responsible for memory formation, storage, and processing, which is commonly impaired in AD patients. The temporal lobe is a region that is greatly affected by AD starting in the earliest stages of AD. The size of temporal lobe gyri, such as middle temporal, inferior temporal, superior temporal, and fusiform gyrus, were found to be significantly different between demented and non-demented subjects in the current study, which is in agreement with previous studies (Visser et al 2002). Amygdala, one of subcortical structures associated with temporal cortex did not a show a significant difference in size between demented and non-demented patients (Figure 4A), which is in contrast with previous studies. However, other subcortical temporal lobe structures such as hippocampus, parahippocampal gyrus, and entorhinal cortex all showed significant differences; these three regions play a major role in memory.

The frontal lobe is responsible for higher level executive functions, expressive language, and movement. The caudal middle frontal gyrus showed no significant difference in demented vs non-demented subjects. The other region in the frontal lobe, the rostral middle frontal gyrus showed a significant difference. The frontal lobe experiences atrophy in later stages of Alzheimer's disease (Scahill et al. 2002), but it is not present in early stages. Another form of dementia called frontotemporal dementia is known to preferentially affect frontal lobes and

temporal lobes. Atrophy in those regions are distinctive indication of frontotemporal dementia, while atrophy in the temporal lobe and hippocampal formation is more typical of AD (Frisoni 1996).

The primary function of the occipital lobe is visual processing. The lateral occipital gyrus is a region that is responsible for object recognition (Grill-Spector et al. 2001). This region has been shown to undergo atrophy in AD patients in late stages of the disease (Yang et. al 2019). Similar to other studies, when compared to non-demented patients, demented patients showed a significantly reduced cortical volume in this region. It has been observed previously that there is a correlation between the atrophy in the occipital lobe and AD when an individual is experiencing visual hallucinations as a symptom (Holoroyd 2000).

Lastly, the insula showed significant decrease in cortical volume in demented patients, which has also been shown in living subjects (Sulimar et al. 2009). Atrophy in the insula have been related with apathy severity in neurodegenerative diseases including as AD (Belkhiria et al. 2020). Apathy is a common symptom in patients with AD (Landes et al. 2001). While, damage where the insula is linked to apathy, this is a region that is also critical for a wide variety of other functions from sensory and affective processing to high-level cognition. These functions carried out by the insular cortex is related to symptoms experienced in AD (Uddin et al 2017).

Overall, we found that the effect of dementia on cortical atrophy in post-mortem brains is congruent with findings reported in-vivo studies (Slumier et al. 2009). The one finding that was in contrast to other studies is the absence of significant atrophy in the amygdala, which was unexpected because many similar studies have found that the atrophy of the amygdala appears in the early stage of AD (Cuénod et al 1993).

Inter-hemispheric differences in atrophy

The four lobes perform slightly different functions depending on the hemisphere. We evaluated cortical atrophy in the corresponding regions of both hemispheres, comparing the left hemisphere samples to the right hemisphere samples. In post-mortem brain samples, the right hemisphere showed significant differences associated with dementia in all regions except the caudal middle frontal gyrus. The regions that showed significant differences in volume between demented and not-demented patients were the fusiform, inferior temporal, middle temporal, and superior temporal gyri. As expected, these were the regions located in the temporal lobe which has shown to be the most effected in AD.

Limitations

This study has several potential limitations. The number of left hemisphere samples was only 20 demented and 20 non-demented patients. To get a better understanding of ex-vivo imaging data trends between hemispheres, performing another study with a larger sample size will be helpful, as it will increase the statistical power and provide a clearer result. Additionally, the current study did not include many healthy and young brain samples. Adding samples from younger individuals as well as healthy controls will provide more complete dataset and will allow to assess age-related changes as well as provide a better understanding of disease-related trends.

Another limitation of this study is that the brains were imaged after removed from the skull, which may have distorted the shape of atrophic brains more than unaffected brains and affected the results. Lastly when analyzing the data, we did not have the ante-mortem MRI data, so we were unable able to compare it to our post-mortem data set.

Implications of this study, conclusions and future directions

The goal of this study was to compare the volumes of various brain regions in demented and non-demented patients using ex-vivo MRI, and also compare the ex-vivo data with in vivo data from other studies. We report, that the volume of the brain and its regions showed trends similar to ones observed in living people. The findings in temporal, occipital, frontal, and the parietal lobe were consistent with previous observations that demented patients experience more atrophy. As expected, the atrophy in the temporal lobe was prominent across all AD stages. A major difference in our findings compared to previous in vivo studies is that we did not observe a significant difference in the volume of the amygdala between demented and non-demented patients. Previous studies showed atrophy in amygdala in the earliest clinical stages of AD (Poulin et al 2012).

. Because AD is a progressive, the brain changes a lot over time. Studies have shown that AD begins years before the symptoms of dementia appear, therefore using structural MRIs and monitoring atrophy of regions will help with early diagnosis. Early diagnosis is very important because not only does this allow the individual and others around them prepare for it, but it allows doctors to help treat the symptoms in the early stages hoping to slow down the disease.

Studying post-mortem brains provides information about the structure and pathology of the diseased brain and allows to better understand the disease pathology and progression. When the individual is diagnosed while living, this diagnosis is based on the different tests and techniques, but it is the patient is stilled considered "probable AD" or "possible AD". The diagnosis can only be confirmed at autopsy, and definitive correlations the MRI findings can only be made post-mortem.

The number of cases of AD is projected to increase over the years. Early diagnosis and preventative measures for AD are critical. Using structural imaging in post-mortem brains will help us understand how cortical atrophy in AD correlates with clinical symptoms and neuropathological findings. This study mostly focused on late stage of AD, because it is the final stage of AD preceding death and such brains are most abundant in brain banks established to study neurodegeneration. Further studies are needed to study the mild and moderate stage of AD, because therapeutic interventions are more likely to succeed when applied early in the diseased process, before neurodegeneration has reached its final stage.

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