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The Role of Olfactory Dysfunction and Cognitive Decline Observed in the Ageing Population, MCI and AD

By

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I would like to thank my supervisor Professor Annalena Venneri for giving me the opportunity to continue my education. I actually benefited a lot in her lab, where there is perseverance and persistence to extract the required information and present it in an appropriate way. She is a source of experience and knowledge which directs us to the right path to reach the other side of the journey. I would also like to thank the second academic supervisor Dr Matteo De Marco, who has perfected touches in the technical and practical part to achieve the desired goal of the research.

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I would like to thank my family for the sacrifices they made for me during this long journey in which we lost relatives whom we were not around because of the reasons for travel and distance. My family has given as much as I did to earn what is better for us. Also, I thank my father who pushed me so much to go on this trip. Every time I decided to stop, he was against it and even urged me to continue. My father is a retired man. He has been a school principal for more than three decades and he appreciates education and loves knowledge and wants us all to continue this love and passion. He taught me a lot, and I dedicate this PhD thesis to him, hoping that it will be even a little proof of what he taught me.

Abstract:

Normal ageing, neurodegenerative diseases and dementia may be overlapping in symptoms and it is confusing to differentiate between them. Olfactory dysfunction is one of the symptoms that may be present in all these conditions. It is difficult to distinguish whether the impaired sense of smell is due to normal ageing or Alzheimer's disease. It is necessary to find concepts that clarify the data and repercussions of olfactory dysfunction and whether it is accompanied by other indicators.

We assessed how common unawareness of olfactory dysfunction is among the elderly. Olfactory quantitative and qualitative tests and measurement of cognitive abilities in the elderly were used. Also, we used MRI to acquire volumetric measurements of the brain of different age groups including young adults, middle-aged or elderly, as well as Alzheimer's disease and mild cognitive impairment patients to assess progression of atrophy in olfactory related brain regions.

As a result, we found that the majority of the elderly people (95%) in the sample had some degree of olfactory dysfunction and importantly they were unaware of it. Also, lower olfactory memory performance was found to be associated with lower levels of cognitive abilities in the elderly. There was atrophy in olfactory-related brain regions in the elderly, and this atrophy was more substantial in patients with probable Alzheimer's disease dementia of both sexes and in patients with mild cognitive impairment in women. Women were also found to be more likely to have deterioration in the size of olfactory-related brain regions, as well as a deterioration in the sense of smell.

This thesis suggests that healthy elderly people should be followed up cognitively and with objective assessment of their sense of smell by conducting sniff tests, rather than using self-reported measures of olfactory abilities. When assessing volumetric neural loss in olfactory related brain regions, significant volume loss was detected in all areas related to odour processing. Overall, these findings suggest that a combination of deterioration in sense of smell, volume loss in olfactory related brain regions and

presence of cognitive decline should alert to the potential presence of a progressive neurodegenerative disease.

Declaration:

I, the author, confirm that the Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not previously been presented for an award at this, or any other, university.

Publications arising from this thesis:

Amal Loudghi, Majed Alotaibi, Melissa Lessard-Beaudoin, Denis Gris, Kate Busch, Pierrette Gaudreau and Rona K Graham. (2019) Unawareness of Olfactory Dysfunction in Older Adults. Int J Neurol Neurother 6:086. doi.org/10.23937/2378-3001/1410086

Al-Otaibi Majed, Lessard-Beaudoin Melissa, Castellano Christian-Alexandre, Gris Denis, Cunnane C. Stephen and Graham K. Rona (2020) Volumetric MRI Demonstrates Atrophy of the Olfactory Cortex in AD. Current Alzheimer Research; 17(10). doi.org/10.2174/1567205017666201215120909

It is worth noting that in the second experiment of the fourth chapter, I was not the examiner for cognitive performance tests (t-MMSE and TICS) among the participants in the study, because it needed specialists in this type of tests, as it requires experience and knowledge of the culture and daily life matters of the country. At that time, I did not have that experience and also the language (other than English) did not support me sufficiently and not as it is now from experience and development in all aspects.

Moreover, in the fourth experiment of the fifth chapter, it was a joint collaboration from my laboratory with another laboratory. I received the raw data, modified, revised, analysed it,

and presented the final results of this study. At that time, I had created a hypothesis regarding olfactory cortex volume but my laboratory didn't have any data of this kind, so we contacted another laboratory and they gave us this data (as ROI extracted volume) that you see in the study.

I was the centre and main source of the data as the Awareness article (experiment 1) is a sub-manuscript generated from the main study which is the ORCA study (experiment 2). I administered the olfaction tests and collect the data and run the analysis tests. However, some of the olfaction tests were administered by others as my proficiency in the French language was not sufficient to facilitate such olfaction tests. I have participated in writing and reviewing the manuscript as well as responding to the reviewers' comments. As it shown in the article, MLB, AL and I wrote the manuscript and run the analysis tests.

Figure 4.1.1: Experimental design of the ORCA study. Was modified and generated from the article.

Table 4.1.1: Descriptive characteristics and summary demographics, smoking status and scores on the UPSIT in the study sample. From the article.

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Figure 4.1.4: Percentage of self-reporting rates on the state of olfaction of participants. Modified from the article.

Figure 4.1.5 The Distribution of reporting rates on the state of olfaction of participants. Not from the article.

In the olfactory cortex manuscript (experiment 4), I was the centre and the main source of the data. I have proposed the study to my direct supervisor as she connected me with another laboratory for possible collaboration. I have received the raw data from this collaborator, checked it, finalised it, run the appropriate model tests and wrote the manuscript as well as responding to the reviewers' comments.

Table 5.1.1. Demographic Profiles. From the article.

Figure 5.1.1: Olfactory cortex and hippocampal atrophy in AD. Generated from the article.

Figure 5.1.2: Select regions of the brain that show hemispheric asymmetry. Generated and modified from the article.

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AAL: Automated Anatomical Labelling; **ACC:** anterior cingulate cortex; **AD**: Alzheimer's disease; **ADRDA:** Alzheimer's Disease and Related Disorders Association; **AFLI:** Adult Lifestyle and Function Interview; **APOE**: Apolipoprotein-E; **APP**: Amyloid precursor protein; **BDNF:** Brain derived neurotrophic factor; **BA**: Brodmann area; **β-Amyloid:** Beta Amyloid; **BMI**: body mass index; **CASPASE:** Cysteine-Aspartic Acid Proteases family; **CBD:** Corticobasal degeneration; **CET:** chronic traumatic encephalopathy; **CNS:** central nervous system; **COVID-19:** Coronavirus disease of 2019; **CRP:** C-reactive Protein; **CSF**: cerebrospinal fluid; **DCX:** microtubule-associated protein Doublecortin; **DS:** Down's syndrome; **FDA:** Food and Drugs Administration; **fMRI**: functional magnetic resonance imaging; **FSL**: FMRIB software library; **FWE**: family-wise error; **GABA:** Gamma-aminobutyric acid; **GFAP:** Glial Fibrillary Acidic Protein; **GM**: grey matter; **GWAS:** genome wide association studies; **HC**: healthy controls; **HD:** Huntington's disease; **IC:** insular cortex; **LDL:** low-density lipoprotein; **MAP;** microtubule-associated protein; **MEG:** magnetoencephalography; **MCI**: Mild cognitive impairment; **MMSE**: Mini-Mental State Examination; **MS:** multiple scleroses; **MNI**: Montreal Neurological Institute; **MRI**: magnetic resonance imaging; **NeuN:** neuronal nuclear protein; **NFT**: neurofibrillary tangles**; NIAA**: National Institute of Aging-Alzheimer's Association; **NINCDS:** National Institute of Neurological and Communicative Disorders and Stroke; **NuAge:** Quebec Longitudinal Study on Nutrition and Successful Aging; **ODMT:** Odour Discrimnation/Memory Test; **OC:** Olfactory Cortex; **OCV:** Olfactory Cortex Volume; **OFC:** Orbitofrontal Cortex; **OFCV:** Orbitofrontal Cortex Volume; **ORCA:** the Olfactory Response Cognition and Aging; **PC:** Piriform Cortex; **PCC**: posterior cingulate cortex; **PD:** Parkinson's disease; **PET**: positron emission tomography; **PHF:** Paired helical filaments; **PiD:** Pick's disease; **POC:** Primary olfactory cortex; **PSEN1:** Presenilin 1; **PSP:** Progressive supranuclear palsy; **SADD:** Sheffield Ageing and Dementia Database; **SNP:** Single Nucleotide Polymorphism; **SPM**: Statistical Parametric Mapping; **SPSS**: Statistical Package for the Social Sciences; **STS**: superior temporal sulcus; **TICS:** Telephone Interview for Cognitive Status; **TIV**: total intracranial volume; **t-MMSE:** Telephone Mini Mantel Statement Examination; **UK**: United Kingdom; **UPSIT:** University of Pennsylvania Smell Identification Test; **USA:** United State of America; **Val66Met:** Valine to methionine substitution at position 66; **VBM**: voxel-based morphometry; **VD**: Vascular dementia; **WM**: white matter;

Chapter 1: Alzheimer's disease overview

1.1 Neurodegenerative disease:

Neurodegeneration refers to the process underlying several conditions that primarily affect the nerve cells (neurons) in the human brain. The brain is a sophisticated clustered organ with a massive number of pathways involved in what is needed for the effective functioning of living beings. The ensemble of neurons constitutes the central nervous system (CNS) that includes the brain and the spinal cord. In the case of neurodegenerative disorders, these neurons progressively lose their ability to function and/or become structurally damaged (de la Monte & Wands, 2004; Gao & Hong, 2008). The process of neurodegeneration varies depending on the type of disorder causing neuronal death. Examples of neurogenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis, Huntington's disease (HD) and more (Doty, 2012; Gao & Hong, 2008; Gitler et al., 2017). Some of the neurodegenerative diseases have obvious symptoms like Parkinson's disease or non specific symptoms like Alzheimer's disease. There are differences between these diseases that depend on the symptoms they cause, on the type of progression and on how the affected patient can live with the disease. However, the diseases have as a common denominator the fact that they affect parts of the CNS and they are age-dependent (Gitler et al., 2017). The cells of the brain are communicating and closely linked, so miscommunications in one

area can disrupt other brain activities and these brain disorders may result in widespread problems.

In their forecast of age-dependent diseases (those affecting elderly people), the United Nations' report on the world population in 2015 indicated that the number of people aged 60 and older worldwide would be more than double in the next 35 years to be approximately 2.1 billion people (Wyss-Coray, 2016). Amongst the various neurodegenerative conditions of the central nervous system that affect the ageing population, one of the most common diseases responsible for progressive cognitive decline in the elderly population is Alzheimer's disease (Alzheimer et al., 1995; Stelzmann et al., 1995).

1.2 Alzheimer's Disease:

Alzheimer's Disease is a progressive neurodegenerative disease that causes deterioration in memory, thinking abilities and behavioural change (Gili et al., 2011; Murphy et al., 2003; Skup et al., 2011). Alzheimer's disease (AD) is the most common type of neurodegenerative dementia, and accounts for about 60-70% of all dementia cases (*Dementia*, 2017; Koca et al., 2017). Alzheimer's disease is one of the world's leading health problems. This health problem is one of the greatest health challenges worldwide. As individuals live longer, the prevalence of age-related neurodegenerative diseases is increasing dramatically, affecting millions of people around the globe. There are 850000 people in the United Kingdom who live with dementia, causing £34.7 billion spent in health care. By 2040, the number of people with dementia will rise to 1.6 million, which may increase costs as high as £94.1 billion, and this rise in the number of people with dementia would increase costs for the NHS, homecare, residential care, and family members (Wittenberg et al., 2019, 2020). Worldwide, 58% of people with dementia live in low and middle income countries, and this percentage will rise to 70% by 2050 (Gulland, 2012). Cognitive decline due to AD is not a normal consequence of ageing but the risk of developing AD increases with age (Guerreiro & Bras, 2015). More than 90% of AD cases manifest and are diagnosed after 65 years of age (Doan et al., 2017). Moreover, the prevalence estimation of Alzheimer's disease is about 3% in the 65-74 years age range, 17% in the 75-84 years age range and 32% in the individuals over 85 years of age (Hebert et al., 2013). At the clinical level, the consequences of AD manifest as impairments in mental and social skills that impede function in daily normal life.

Alzheimer's disease causes brain atrophy consequent to the death of brain cells that leads to a continuous decline in memory and overall mental abilities. To detect global changes in mental abilities, screening tools such as for example the Mini Mental State Examination (MMSE) (Folstein et al., 1975) can help physicians if they have a concern that a patient might be developing the disease. This and other screening measures are designed to assess the global cognitive status of the patient (Butterfield et al., 2013). These screening tools, however, are not diagnostic instruments and they have to be supplemented by extensive neuropsychological assessment, assays of cerebral spinal fluid to look at disease biomarkers and neuroimaging, especially magnetic resonance imaging (MRI) to detect global or regional brain shrinkage (Butterfield et al., 2013; Dubois et al., 2014).

In addition to ageing, AD risk factors include the presence of the Apolipoprotein E allele ε4 (*APOE ε4*) that is a major genetic risk factor for the sporadic late onset form of the disease (Albert et al., 2011; Hennebelle et al., 2015; Lloret et al., 2015). Additional risk factors include family history, a low level of physical activity (Cermakova et al., 2015; Hennebelle et al., 2015), high cholesterol levels and a low level of education (Cermakova et al., 2015). However, not every individual who has one or more of the established AD risk factors will have the illness (Figure 1.2.1).

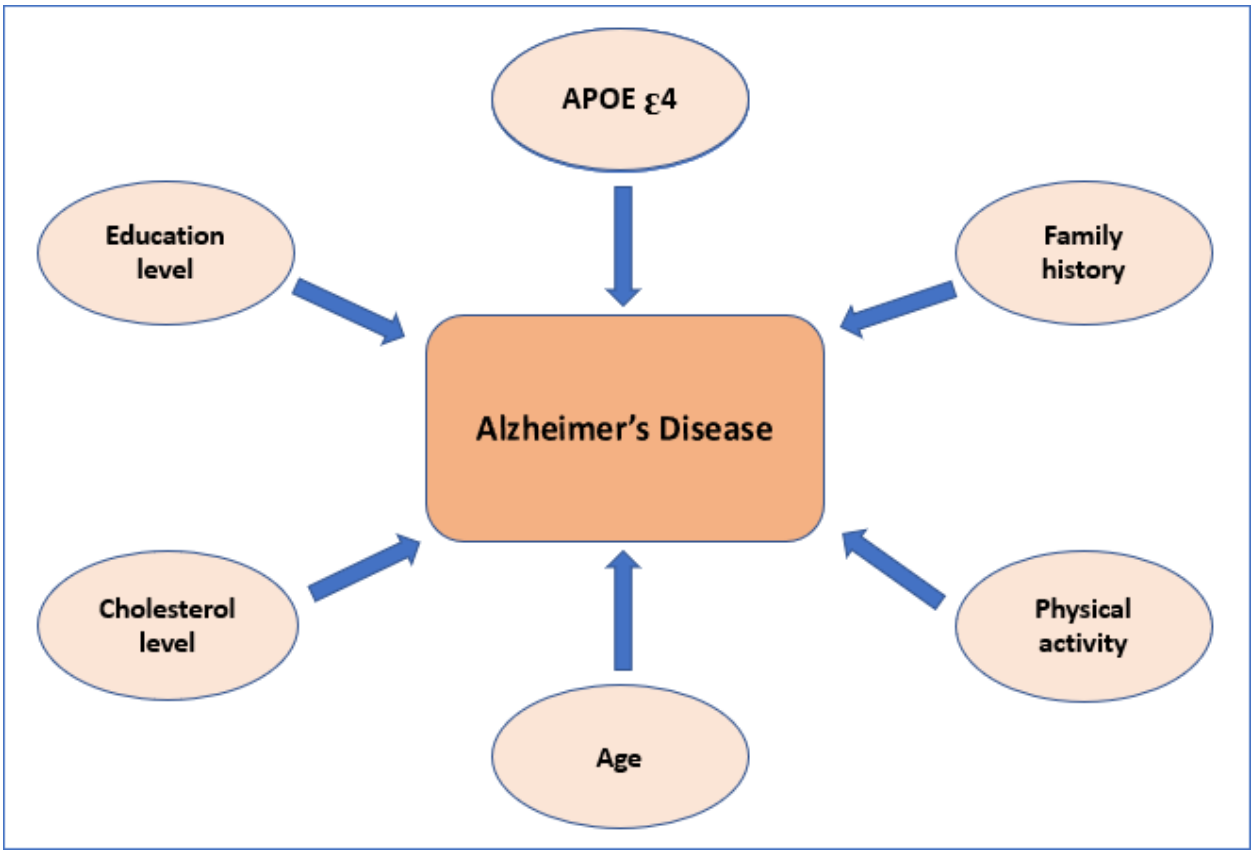


Figure 1.2.1: AD risk factors. Many risk factors may lead to developing AD including ageing, *APOE ε4*, family history, low physical activity, high cholesterol level and low education level.

1.2.1 Alzheimer's Disease first case

In 1906 the first description of this disease referred to Alzheimer's Disease (AD) as a "peculiar disease" (Boughey & Graff-Radford, 1987; Snowdon, 1997). It was an undescribed disease at that time when the German physician Alois Alzheimer observed a new condition experienced by patient August D, whose collection of symptoms was at the time completely unknown to clinicians (Hippius & Neundörfer, 2003). The patient showed a profound loss of memory, unfounded beliefs about her family (e.g. a delusion of jealousy towards her husband) with other psychological changes as well as shrinkage and alteration in the nerve cells observed at the time of brain autopsy (Möller & Graeber, 1998; Small & Cappai, 2006). The new disease was named by Emil Kraepelin after the physician who reported the first case. At the time no one knew that this disease will be one of the most important and famous diseases that affects millions of people worldwide and unfortunately it remains still without an effective form of treatment to date (Yilmaz et al., 2017). Most importantly, AD still remains one of the most common and serious neurodegenerative disorders in humans.

1.2.2 Type of Alzheimer's disease

There are two types of Alzheimer's disease: sporadic AD and familial AD (Jouanne et al., 2017). The sporadic form of AD is the most common type while the familial form of AD accounts only for a small percentage of all AD cases (Hatami et al., 2017). Sporadic

AD is known as late-onset AD (Hatami et al., 2017) while early-onset or familial AD is associated with mutations in certain genes such as Presenilin 1 and 2 (Hatami et al., 2017; Muratore et al., 2014; D. Xia et al., 2015) and in the amyloid precurson protein (APP). These genetic mutations occur on chromosomes 21, 14, and 1 (Heininger, 2000). The sporadic form of AD is found to be associated with age and it is more frequently observed in the elderly population, i.e. 65 years and older. However, people of younger age might be affected as well.

1.2.3 Stages of Alzheimer's disease

The progression of the disease has been described by Braak and Braak, 1991. Based on evidence from pathological progression, six disease stages have been established according to changes in neurofibrillary tangles and neuropil threads, and amyloid depositions (Alafuzoff et al., 2008; Braak & Braak, 1991; Wischik et al., 2016). However, the disease has no well recognised clinical diagnostic framework since some have classified AD as a three stage condition, namely early, middle and late stage, or some consider the three stages as pre-symptomatic, prodromal (MCI) and dementia (Dubois et al., 2014; Jack et al., 2010; Koca et al., 2017), a framework that is universally adopted, and some as a four stage condition, namely preclinical AD, Mild Cognitive Impairment (MCI), early AD and late stage AD (Butterfield et al., 2013). Clinical diagnosis is reached by combining laboratory results with a physician clinical judgment by following a specified set of either clinical or research criteria (Jack et al., 2018; McKhann et al.,

2011). Commonly a three stage classification of AD is widely used in the literature and these will be described in more detail in the following section (sign and symptom).

1.2.4 Signs and symptoms of AD

Different signs and symptoms can be found at onset and these can be very mild to begin with, but gradually they will increase with the progression of the disease. Neurological and behavioural symptoms used to identify the disease include memory deterioration manifesting as a disturbance in acquisition and retention of new memories, partial linguistic dysfunction, difficulties in dealing with multi-tasking, accelerated forgetting, attention disturbances, functional deficits, including failure to do simple daily tasks, but also behavioural symptoms such as anxiety, depression and lack of motivation (Donovan et al., 2018; Morley et al., 2018).

The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984; McKhann et al., 2011) classifies AD in three clinical stages as probable, possible and definite Alzheimer's disease. The clinical criteria for probable Alzheimer's disease diagnosis include: (1) dementia diagnosed by clinical examination by the Mini-Mental State Examination, Blessed Dementia Scale, or some other similar screening scales, and confirmed by more extensive neuropsychological tests; (2) deficits in two or more areas of cognition; (3) progressive deficits in memory and other cognitive

functions; (4) no disturbance of awareness; (5) age between 40 to 90 years and often after 65 years old; (6) no other brain disease or systemic disorder that can be the cause of progressive deficits in memory and cognition. Also, (McKhann et al., 1984; McKhann et al., 2011) clinical criteria for possible Alzheimer's disease diagnosis include: (1) may be made in the presence of a second systemic or brain disorder which is sufficient to produce dementia but which would not be classified as the cause of dementia. (2) when the individual shows progressive severe cognitive decline gradually, in the absence of other identified causes, leads to a diagnosis of probably Alzheimer's disease. The third criterion of (McKhann et al., 1984; McKhann et al., 2011) for a definite Alzheimer's disease diagnosis includes: (1) when the criteria for probable Alzheimer's disease are met and (2) there is histopathological evidence confirmation from biopsy or autopsy.

1.2.5 Risk factors:

1.2.5.1 Cognitive decline

In the literature, some degree of cognitive decline or change is considered as a normal part of ageing since some cognitive abilities such as memory (especially episodic memory) would decline gradually with time (Harada et al., 2013). Cognition is an essential component of the human ability to communicate and survive and when this changes, it could be harmful to the individual and to those they will be in contact with.

However, at times there is a mild decline that appears to exceed what would be expected in relation to ageing; mild cognitive impairment is considered as a pre-dementia stage of AD (Morris et al., 2001) and confers an increased risk for progression to dementia. (Petersen, 2004) defined people who experience Mild Cognitive Impairment (MCI) as individuals who have some cognitive impairment but are of milder severity to be diagnosed as dementia. The most important concept of this criterion is that the person, although experiencing some impairments of cognition, still retains the ability to function well in daily life. The MCI diagnostic criteria devised at the Mayo Clinic Alzheimer's Disease Research Center/Alzheimer's Disease Patient Registry mainly focus on memory impairment with less focus on impairments of other cognitive domains. These criteria include: (1) memory complaint, preferably supported by an informant, (2) impairment in objective memory for age, (3) relatively preserved general cognition for age, (4) essentially intact daily living activities (5) no dementia.

Some MCI patients show mild cognitive deficits in various aspects of cognition, but their performance is not sufficiently severe or widespread enough to meet the criteria for dementia (Belleville et al., 2017; Petersen, 2000). Episodic memory, associative memory, memory recollection, semantic memory and working memory are impaired in MCI (Belleville et al., 2014).

1.2.5.2 Ageing

Ageing is the highest risk factor for AD (Guerreiro & Bras, 2015; Niccoli & Partridge, 2012). Ageing causes a reduction in physiological functioning and is also a risk factor for many diseases. It is the risk factor most associated with AD (Xia et al., 2018). In ageing, we may have health problems such as high blood pressure, heart conditions and decline in the immune system. In the United State, the percentage of patients with AD increases with age from 3% in the age range between 65-74, to 17% in the age range between 75-84 and to 32% in the age range between 85 and above (Hebert et al., 2013). There is a very thin line between the changes caused by ageing and those caused by AD in the brain. However, unlike in AD, in normal ageing neurons count does not change significantly (Xia et al., 2018). A variety of health problems, however, occurs with ageing including cognitive decline, cardiac disorders and neurodegenerative diseases (Cermakova et al., 2015), all increasing the risk of AD.

1.2.5.3 Genetics

There are genetic risk factors and causes of the disease that are extensively known now. These include the Apolipoprotein E (*APOE*) ε4 allele conferring an increased risk and mutation of genes involved in the Amyloid-β and Tau proteins that are genetic determinants of this disease. However, there are more than 20 identified variants in genes that can modulate risk in AD or cause AD such as presenilin 1 (PSEN1) and the amyloid-β precursor protein APP (Guerreiro et al., 2013; Harold et al., 2009; Karch & Goate, 2015).

Most of the literature classifies AD as a non-genetic disease. However, some studies have used a polygenic risk score to show how genetics can predict sporadic AD. This method uses genetic data to illustrate the risk score of the prediction of the disease compared with control. It calculates the genetic variants based on information obtain from a level as small as a Single Nucleotide Polymorphism (SNP) or as large as from genome wide association studies (GWAS). Genome-wide association studies have identified approximately 40 SNPs associated with AD (Baker & Escott-Price, 2020). (Escott-Price et al., 2017) indicated that polygenic risk factor analysis is a reasonable method of AD prediction and could also be useful in clinical practice. Moreover, the calculation of a polygenic risk score can be used to identify MCI individuals at risk of progressing to AD dementia (Chaudhury et al., 2019).

1.2.5.4 Lifestyle

Some of the AD risk factors are fostered by individual actions or habits individuals and society may adopt and some lifestyle choices may trigger or accelerate the development of AD (Pope et al., 2003). Physical activity can improve cognitive functioning (Lautenschlager et al., 2008; Smyth et al., 2004), while a sedentary life may have a negative impact. Evidence suggests that physical exercise has a positive influence on cognitive functioning among the elderly population and in AD patients and may help in reducing the risk of development of dementia in AD (Castellano et al., 2017; Langlois et al., 2013). The evidence is not unanimous, as in the elderly, doing intense physical activity might be harmful to the body and some studies have not found a benefit on cognition (Faber et al., 2006; Kramer et al., 1999). There is, however, more recent evidence from longitudinal interventional studies that suggests that exercising, in the context of a multidomain intervention programme, may be beneficial and lower dementia risk in the elderly (Ngandu et al., 2015). This 2 year multidomain intervention (diet, exercise, cognitive training, vascular risk monitoring) study found that there are significant beneficial effects in a change in overall cognition, executive function and processing speed as well as other aspects such as dietary habits, and physical activity. By modifying lifestyle, therefore, a reduction in the burden of disease can be achieved individually and globally. Interestingly, a healthy diet has been found to be effective in reducing the development of several diseases including AD (Pope et al., 2003).

Other lifestyle factors are involved in the development of AD or interact with the pathological pathways of the disease. Some of the lifestyle factors that may increase the risk of AD are low education, being cognitively inactive, high cholesterol, being overweight or obese and having diabetes (Mortimer et al., 1991; Ott et al., 1999; Peila et al., 2002; Pope et al., 2003; Stamler, 2000).

1.2.6 Brain alteration

1.2.6.1 Amyloid beta and Tau proteins in AD

The typical Alzheimer's disease histopathological hallmarks are the presence of deposits of β-Amyloid (senile plaques) and aggregation of Tau protein (neurofibrillary tangles) (Butterfield et al., 2013; Chu et al., 2017; Eriksen et al., 2003; Gong & Iqbal, 2008; Mandelkow, 1998; Mota et al., 2015; M. P. Murphy & LeVine, 2010; Perl, 2010). There are other neuropathological lesions detected in AD but the two above are universally recognised as characteristic of this disease. Scientists believe that those two pathological features are the most well known alterations that lead to AD (Brier et al., 2016; Jouanne et al., 2017; Murphy & LeVine, 2010). However, it is still a matter for debate which pathological feature is crucial in determining the disease. In the case of senile plaques, the β-Amyloid protein is derived from the Amyloid Precursor Protein (APP) which is a transmembrane glycoprotein (Murphy & LeVine, 2010; Perl, 2010). When an alteration occurs in this pathway, the β-secretase enzyme cleaves APP to release a large fragment and the remaining part of APP is cleaved by ɣ-secretase to generate β-Amyloid (Eriksen et al., 2003; Murphy & LeVine, 2010) (figure 1.2.2). The creation of neurofibrillary tangles is a result of the aggregation of intraneuronal hyperphosphorylated tau (Israel et al., 2012). Some studies indicate that β-Amyloid plaques are followed by phosphorylated Tau aggregation *in vitro* (Lloret et al., 2015). Importantly, Chabrier et al. (2012) have shown in a transgenic AD mouse model that the reduction in β-site APP cleaving enzyme decreases soluble β-Amyloid and reduces Tau accumulation and phosphorylation (Chabrier et al., 2012). However, it is unclear whether the level of β-amyloid directly

causes increases in the level of phosphorylated Tau (Israel et al., 2012). Low Amyloid-β42 in the cerebrospinal fluid (CSF) has been found to be a sufficient biomarker of AD together with the increase in hyperphosphorylated Tau protein (De Vos et al., 2016; Henriques et al., 2018).

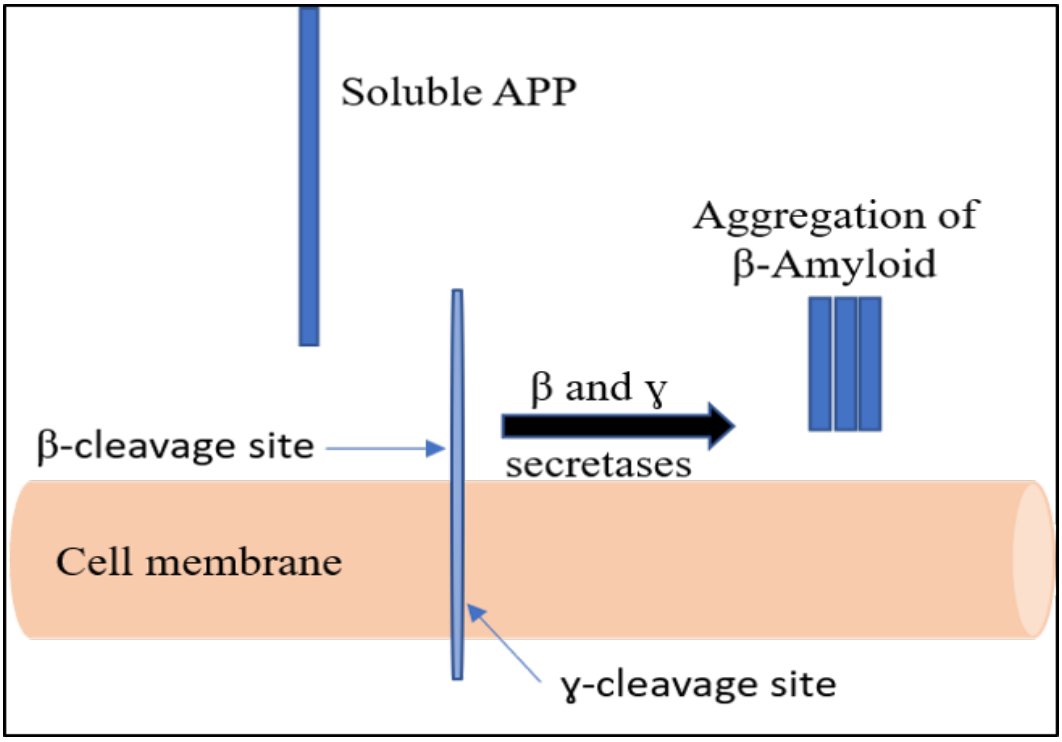


Figure 1.2.2: The cleavage site of the Amyloid Precursor Protein: In the case of AD, the two proteases (β-secretase and γ-secretase) are involved in the pathological pathway of the release of the toxic Beta-Amyloid as β-secretase cleaves APP to release a large fragment of APP and γ-secretase cleaves APP and releases a toxic small fragment of β-Amyloid.

Tau normal function:

Proteins are essential and present in every cell and they are responsible for many functions in the human body. Proteins function in the cells include support, stabilisation, transmission, replication and many more. The cytoskeleton of the eukaryotic cells is a network of filamentous polymers and regulatory proteins and it has a dynamic and

adaptive structure (Fletcher & Mullins, 2010). The cytoskeleton consists of three main polymers: microtubules, intermediate filaments and actin filament (Fletcher & Mullins, 2010; Köster et al., 2015). In addition to shaping the neurons architecture, the cytoskeleton plays a major role in the functions and the characteristics of the neurons such as regulation of plasticity and synapse formation (Cárdenas et al., 2012). Thus, it is critical that the microtubules remain stable to provide normal processing and functions. The Tau protein is a microtubule-associated protein (MAP) that works as a stabiliser of neuron microtubules (Harada et al., 2016; Israel et al., 2012; Shukla et al., 2012) and it makes the paired helical filaments (PHF) (Butterfield et al., 2013; Künzi et al., 2002; Mandelkow, 1998). Alteration in microtubule stabilisation may lead to pathological disturbance in the normal structure and functions of the cytoskeleton.

Tau structure:

The Tau gene in the human body is found in the long arm of chromosome 17 as (17q21) (Cárdenas et al., 2012; Gong & Iqbal, 2008); it contains 16 exons and encodes six tau isoforms in the adult human brain (Goedert et al., 1989; Gong & Iqbal, 2008). Those six isoforms are different from each other by the presence or the absence of one or two inserts (amino acids 29 or 58) in the N-terminal and by the presence of three or four repeats in the C-terminal part (Gong & Iqbal, 2008). Importantly, the repeats in the C-terminal half are the domains to which tau binds to the microtubules (Goode & Feinstein, 1994; G. Lee et al., 1989). Each isoform has its own physiological and biological differentiation depending on the characteristics of the structure and its position. Thus, Tau deposition shows complicated characteristics depending on those isoforms.

Regions where Tau is expressed:

By knowing which brain regions might be more likely to express Tau deposition, it would be easier to identify the starting point of this alteration in the brain. It is critical to illustrate those regions where the distribution of altered Tau is located to help forming a better understanding and prevention procedure. The shortest isoform of tau is expressed in the foetal brain while all the other six isoforms are expressed in the adult brain (Kosik et al., 1989; Stanford et al., 2003). There is an abundance of Tau protein in the neurons in the Central Nervous System (CNS) while very low levels of Tau are found in the astrocytes and in the oligodendrocytes (Šimić et al., 2016). In the neurons, the six isoforms of Tau are most likely found in the axons (Jouanne et al., 2017; Shukla et al., 2012). Neurofibrillary tangles are composed of the aggregation of intracellular hyperphosphorylated Tau protein (Lee et al., 2010).

Tissue damage:

Tau deposition related damage starts in the entorhinal cortex and then spreads to the hippocampus, frontal and temporal cortices and eventually to all areas of the isocortex (Hu et al., 2017). The spread of Tau alterations leads to synaptic disorder, glial activation resulting in neuronal loss (Harada et al., 2016).

Tau in other diseases:

There are other neurodegenerative diseases that are characterised by the presence of filamentous Tau such as Down's syndrome (DS), corticobasal degeneration

(CBD), progressive supranuclear palsy (PSP), Pick's disease (PiD) (Abraha et al., 2000; Hu et al., 2017) and chronic traumatic encephalopathy (CTE) (Harada et al., 2016).

1.2.6.2 Atrophy:

Brain atrophy is an important detectable sign of AD and is used in the diagnosis of the disease. However, there are important differences in the progression of atrophy between men and women as in the hippocampal region of the brain, women have faster progressing atrophy than men (Mazure & Swendsen, 2016). In the clinical diagnosis of AD, the volume of the left hippocampus is used for identifying the stage of the disease (Uysal & Ozturk, 2020). Right hippocampal atrophy is instead associated with a more advanced stage of AD (Lee et al., 2019). In terms of brain atrophy, the influence of age in AD patients is greater than normal ageing effects (Fiford et al., 2018). In AD, neuronal death and tissue loss lead to atrophy in most brain regions. A study showed an increased rate of ventricular expansion and global brain atrophy in AD patients (Schott et al., 2005). As MRI scanning is safe, MRI can track brain changes caused by the distribution of amyloid and Tau pathology that follows distinct patterns and this distribution allows the identification of the different AD stages (Belleville et al., 2014). Studies indicate that measures of hippocampal volume or of the medial temporal complex volume can predict progression from MCI to AD (DeCarli, 2007; Fleisher et al., 2008), but there might be some overlap with normal ageing. However, Fleisher et al, (2008) found that measures of hippocampus volume can predict conversion from MCI to AD with a 60% accuracy and this accuracy rises to 78.8% when combined with clinical data (Fleisher et al., 2008). MRI

can discriminate AD from healthy participants with high sensitivity and specificity (McEvoy & Brewer, 2010). McEvoy and Brewer, (2010) state that studies show that quantitative MRI measures are sensitive to the neurodegeneration that occurs in prodromal and established AD and suggest that structural MRI can be used to detect AD prior to the onset of dementia (McEvoy & Brewer, 2010).

1.2.6.3 Tissue inflammation:

In addition to the presence of senile plaques and neurofibrillary tangles, indicating abnormal amyloid and tau protein deposition in the brain, neuropathological studies have also reported strong evidence of inflammatory pathways' activity in the AD brain. Cellular and molecular inflammatory markers are observed in the pathology of the disease manifesting as an increased number of activated microglia and astrocyte cells (Eriksen et al., 2003; Heneka et al., 2015; Lee et al., 2010; Wyss-Coray & Rogers, 2012). During neuroinflammation, failure of synapses to express plasticity may lead to cognitive impairment (Mancini et al., 2017). Some of these inflammatory processes are protective and some are harmful. Some markers are used to study inflammation such as IBA-1 that visualises microglia cells (Ohsawa et al., 2004). Those microglia cells can be used as indicators of the occurrence of any tissue inflammation (Citron et al., 2008; Mandrekar-Colucci & Landreth, 2010). In research, tissue staining is a well recognised method for the detection of tissue inflammation.

1.2.6.4 Neurogenesis:

This term refers to the process of formation of neurons and this endogenous procedure includes proliferation, differentiation and migration of the new neurons (Eriksson et al., 1998; Shohayeb et al., 2018). Formation of mature neurons from neural stem cells is called adult neurogenesis (Abdissa et al., 2020). Some researchers do not believe there is such a way of regenerating cells inside the brain while others support the idea that neurogenesis occurs. In animal models, it has been found that there is the production of new neurons in the hippocampus and it is called adult hippocampus neurogenesis. Adult hippocampus neurogenesis in humans, for example, was first shown by Eriksson et al 1998 who suggested that it persists in human adults till the ninth decade of life (Eriksson et al., 1998; Moreno-Jiménez et al., 2019; Tobin et al., 2019). This process has been found to be altered in AD (Lazarov & Marr, 2010). It is a normal consequence that degeneration of neurons in the hippocampus results in memory deterioration. Normally, neural progenitor cells give rise to new nerve and glia cells in the hippocampus, olfactory bulb, forebrain subcortical white matter, specific areas in the temporal lobe and more (Lazarov & Marr, 2013). Moreno-Jimenez et al, 2019 state that in AD, alterations in adult hippocampus neurogenesis are observed in the early stages of the disease even prior to the appearance of the disease strongest hallmarks (senile plaques and neurofibrillary tangle). Using immunohistochemistry, this study provided evidence of impairment in neurons' maturation as AD advances as detected by reduction in the expression of Prospero homeobox 1, NeuN and more (Moreno-Jiménez et al., 2019). Deficits in neurogenesis may promote cognitive decline in AD or worsen it and

there is evidence that a level of active neurogenesis is associated with better cognitive diagnosis (Tobin et al., 2019). A neurogenesis dysfunction is said to play a major role in the acceleration of AD pathophysiology (Choi & Tanzi, 2019). In fact, the mutations associated with AD that cause alterations in the metabolic or enzymatic pathways may also alter neurogenesis (Lazarov & Marr, 2013). Different markers have been found to be useful tools to study the process of neurogenesis in AD and these include DCX, NeuN and GFAP (J. Zhang & Jiao, 2015). To detect neurogenesis, different types of assay have been used including thymidine analogue bromodeoxyuridine assay, radioactive and immunohistochemistry markers such as Phosphohistone-h3 (Abdissa et al., 2020).

1.2.6.5 Caspase activation:

Enzymes are biological molecules found in the body involved in acceleration and participation in chemical and biological reactions. There are different families of enzymes depending on their reactions. A group of enzymes that can cleave proteins are called protease that has a Caspase group who can be apoptotic and trigger the cell death program (Florentin & Arama, 2012). The Cysteine-Aspartic Acid Proteases family (CASPASE) plays an apoptotic role in AD as it programs cell death (Chu et al., 2017). Cysteine-aspartic proteases (Caspase) are classified in three groups based on their sequence and function as group I (1, 4 and 5), group II (the executioners 3, 6 and 7) and the initiators group III (8, 9 and 10) (Julien & Wells, 2017). These intracellular caspases are either initiators or executioners and may cleave each other or their substrates (Graham et al., 2011). Riechers et al. (2016) state that studies of patients, animals and

cell culture models provide sturdy proof of potential universal mechanisms of caspases within the early stages of pathways resulting in neuronal death in AD (Riechers et al., 2016). A recent study showed that there is an association between levels of expression of active caspase 6 and lower memory ability, lower global cognition, and poorer semantic memory and MMSE scores (Foveau et al., 2016). These caspases play a role in the loss of neurons in AD (LeBlanc, 2013). A massive effort has been made in research to investigate the possibility of reducing or deactivating these caspases by finding the opposite trigger leading to their activation.

1.2.6.6 Neurotransmitters alteration:

In AD, there is reduction in levels of several neurotransmitters' expression in the brain. The neurotransmitters are chemical molecules that transfer signals among brain cells. When the nerve cells start firing signals among them, neurotransmitters are involved in carrying these signals. They work as messengers or coordinators between the presynaptic terminal and postsynaptic dendrite helping the synaptic process. These neurotransmitters can be excitatory such as Glutamate or inhibitory such as GABA (Gamma-aminobutyric acid) and enkephalins.

Deficits in neurotransmission based on reduction of the level of neurotransmitters such as acetylcholine, amino acids and monoamines are found in AD (Nam et al., 2020; Savelieff et al., 2019; Snowden et al., 2019). This deficit affects brain performance. For example, a low level of acetylcholine leads to cholinergic deficits which affect the neural

system involved in learning and recalling new information and cognition more generally (Lanari et al., 2006; Nam et al., 2020). It becomes increasingly harder for neurotransmitters to perform their task as Amyloid-β aggregates and may put more burden when it binds to metals in the AD brain (da Silva & Ming, 2007; Nam et al., 2020; Parsons et al., 2013). GABA receptors are also altered in AD and there is a compensatory increase in these receptors, within neurons (Iwakiri et al., 2006; Kandimalla & Reddy, 2017). Furthermore, the serotonin (5-hydroxytryptamine) neurotransmitter which is involved in pain, emotions and other aspects of cognition also show decreases in its receptors in AD (Reynolds et al., 1995; Rodríguez et al., 2012). Some neurotransmitters are involved in regulating the sleep-awake state and this may explain why 25-60% of AD patients also experience sleep disorders or abnormalities (Van Erum et al., 2019).

1.2.7 Alzheimer's disease treatment:

The pharmacological treatment of AD involves two categories of intervention: symptomatic treatment such as acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists or aetiology based treatment as amyloid binding and tau banding therapies (Mendiola-Precoma et al., 2016). Symptomatic treatment addressing deficits in neurotransmission plays a major role in current AD therapeutics. Inhibitors are used to slow down the clinical progression of symptoms. Acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine are approved for the treatment of patients with mild AD (Kandimalla & Reddy, 2017). These acetylcholinesterase inhibitors help

cognitive function in mild-moderate, moderate-severe AD patients (Kandimalla & Reddy, 2017; Wang et al., 2015) as they increase the availability of acetylcholine at the synaptic level in the AD brain (Hampel et al., 2018). Aetiology based treatment targets the amyloid cascade by either targeting amyloid binding (by inhibiting or modulating secretase) or preventing aggregation of Amyloid-β or neurofibrillary tangles (Mendiola-Precoma et al., 2016). A form of amyloid binding treatment, aducanumab (a human monoclonal antibody), has recently approved by the United States Food and Drugs Administration (FDA) for the treatment of MCI and early-onset AD and this form of treatment acts by preventing binding and fostering clearing of accumulation of Amyloid-β (Beshir et al., 2022).

AD affects primarily the oldest part of the population and those individuals most likely experiencing a range of other health problems such as high cholesterol levels, diabetes mellitus, high level of low-density lipoprotein (LDL) and hypertension, factors that all may contribute to increase the risk of AD. Thus, medication for these comorbid conditions such as statins or drugs for type two diabetes mellitus (Amylin) may all help lower the risk of AD and have a protective effect in AD (Dias et al., 2015; Godyń et al., 2016; Lin et al., 2015; Mendiola-Precoma et al., 2016).

Chapter 2: Olfactory dysfunction

2.1 Definition of sense of smell

The sense of smell is a chemical sense that gives living beings the ability to recognize specifically molecules of different substances and gives organisms rich and vital information about their surroundings. Functional integrity of the sense of smell is fundamental to the continuity of animals and their struggle for survival. The olfactory sense is critically important for safety. Like other senses, the sense of smell is very important as it can be the first to provide warning signs in many dangerous situations. In addition to the ability of the sense of smell to distinguish dangerous accidental situations in a person's surroundings, such as a fire, or a gas leak in the house, it is also important in influencing a person's mood, as pleasant smells may bring happy feelings while unpleasant smells may disturb a person as well as their enjoyment of food (Attems et al., 2015). The sense of smell has also been said to play a major role in personal taste, behaviour and interpersonal relationships (Sarafoleanu et al., 2009). Moreover, olfaction works along with other senses such as, for example, vision to provide emotion and quality to these sensations (Sarafoleanu et al., 2009; Zador & Mombaerts, 2007).

2.2 Regions of the olfactory system in the brain

Olfactory brain regions play a major role in transforming and translating an odour so that it can be read and recognised. The olfactory system in the brain includes some

regions that have as their only role that of being involved in olfaction and others that are only partially involved in olfaction, but they are also associated with other processes and tasks in the brain. These regions together are involved in a range of olfaction related functions such as odour identification, recognition, discrimination and memory. All these functions require the contribution of different brain regions that participate in the processes that result in accurate sense of smell. The regions that are involved in olfaction processes in the brain include the olfactory bulb, the olfactory epithelium, the olfactory cortex (that includes the piriform cortex), the hippocampus, the parahippocampus, the amygdala, the orbitofrontal cortex and the entorhinal cortex, with the latter five regions being involved in other aspects of brain functioning as well (Firestein, 2001; Karunanayaka et al., 2014; Kubota et al., 2020). Each of these regions does support olfaction for a specific function depending on the specific processing requirements. Specifically, from the olfactory bulb, projections go to olfactory-related brain structures to perceive and interpret odours, but the specific contribution of these individual regions to olfaction processing is still not fully understood (Gottfried et al., 2002). Most of these regions are vulnerable to the effects of normal ageing. Thus, sensory neurons that work as odour selective receptors and first order neurons in the olfactory system decrease in number with ageing (after 65 years of age) (Attems et al., 2015; Rawson, 2006).

Currently, the literature does not contain precise definitions of the olfactory brain regions included in the primary olfactory cortex, the secondary olfactory cortex and/or the olfactory-related cortex. The sense of smell requires sophisticated processing in many brain regions. The regions' role within the overall brain dynamics is not fully understood

as even the definition of olfactory primary cortex is varied in neuroimaging research parcellation studies (Fjaeldstad et al., 2021). A meta-analysis study conducted by Seubert. et al, in 2013 analysed all published olfactory neuroimaging data. The findings indicated that the complete olfactory system in not fully mapped (Seubert et al., 2013). This study identified as olfactory cortex those regions receiving direct projections from the olfactory bulb that include the piriform cortex, anterior olfactory nucleus, olfactory tubercle, anteromedial part of entorhinal cortex, periamygdaloid cortex and several areas within the amygdala (Carmichael et al., 1994; Price, 1985; Seubert et al., 2013). This olfactory cortex regions, therefore, receive direct signals from the olfactory bulb about specific odorants. Anatomically, the olfactory cortex includes the piriform cortex (also called pyriform or prepyriform cortex) that is the largest of its subregions (Wilson, 2009). Another study classified the primary olfactory cortex regions and these included the piriform cortex and closely associated areas of the anterior olfactory nucleus, the anterior perforated substance, olfactory tubercle, anterior portion of periamygdaloid cortex and amygdala (Wang et al., 2010). Doty (2017) defined the primary olfactory cortex as regions that receive projections from the olfactory bulb; these regions include the anterior olfactory nucleus, pyriform cortex, periamygdaloid and amygdala complex regions and rostral entorhinal cortex (Doty, 2017). Olfactory sensory neurons in the olfactory epithelium project to the first olfactory processing region that is the olfactory bulb, and then this region sends information through the olfactory tract to other olfactory cortex regions that contain the piriform cortex, anterior olfactory nucleus, olfactory tubercle, amygdala and entorhinal cortex (Bitter et al., 2010; Gottfried, 2006; Shepherd, 2006).

These areas are connected to the secondary olfactory centres such as the hippocampus, parahippocampus and orbitofrontal cortex in the limbic system (Bitter et al., 2010; Fjaeldstad et al., 2017). In the frontal cortex, the orbitofrontal cortex in the posterior ventral regions receives projections from regions such as the amygdala, piriform cortex and entorhinal cortex (Doty, 2017).

2.3 Olfactory processes

Olfaction processes require emotional and memory-related brain regions. During the smelling process, parts of the brain related to the sense of smell are activated such as olfactory cortex regions, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex (Fjaeldstad et al., 2021; Stadlbauer et al., 2016). Stadlbauer et al, (2016) studied olfactory stimulation in healthy participants using magnetoencephalography to measure brain activity during odour perception; these authors measured activation in the olfactory cortex, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex (Stadlbauer et al., 2016). Moreover, an fMRI study found similar results with activation in these same brain regions during odorant stimulation (Poellinger et al., 2001). Because part of the amygdala receives projections from the olfactory bulb, it is also considered as part of the olfactory cortex (Fjaeldstad et al., 2021). The amygdala is located rostrally next to the hippocampus in the anterior part of the medial temporal lobe (McDonald & Mott, 2017). For olfactory memory, connections are established between the amygdala and the hippocampus and

parahippocampus (McDonald & Mott, 2017). The entorhinal cortex sends information including mnestic information processed by the hippocampus to the neocortex (McDonald & Mott, 2017). During odorant processing, fibres branch to the hippocampus, parahippocampus, amygdala and orbitofrontal cortex (Yousem, et al., 1999). Olfactory-related regions are connected and work at the same time. For example, medial and posterior OFC have high connectivity with olfactory cortex, hippocampus and parahippocampus as the posterior border of OFC lies next to the olfactory cortex while the anterior lateral OFC has moderate functional connectivity with the olfactory cortex, hippocampus and parahippocampus (Du et al., 2020). In fact, different regions in the OFC respond to different types of odorants. In fMRI studies, the medial OFC has greater activation to pleasant odours while the lateral OFC is more activated by unpleasant smells (Anderson et al., 2003; Rolls et al., 2003). Odour identification processing occurs as the chemicals are transmitted from the olfactory bulb to the entorhinal cortex that has projections directly to the hippocampus (Iizuka et al., 2021; Lavenex & Amaral, 2000). The process continues by recognising the odour, by naming and recalling the memory of the odour, then starting neural connections between the hippocampus and parahippocampus with OFC, the role of which in this process is that of odour recognition (Iizuka et al., 2021; Rolls, 2001). The parahippocampus processes information that is then sent to the hippocampus via the entorhinal cortex (Naya, 2016). Odour memory retrieval and emotional reaction to an odorant is mediated by the hippocampus and the amygdala (Kubota et al., 2020; Rolls, 2001). The connection of olfactory-related regions may influence the connected regions negatively. A study showed that the reduction of

entorhinal cortex volume was not directly linked with an olfactory deficit but olfactory impairment correlated with parahippocampus volume loss (Iizuka et al., 2021).

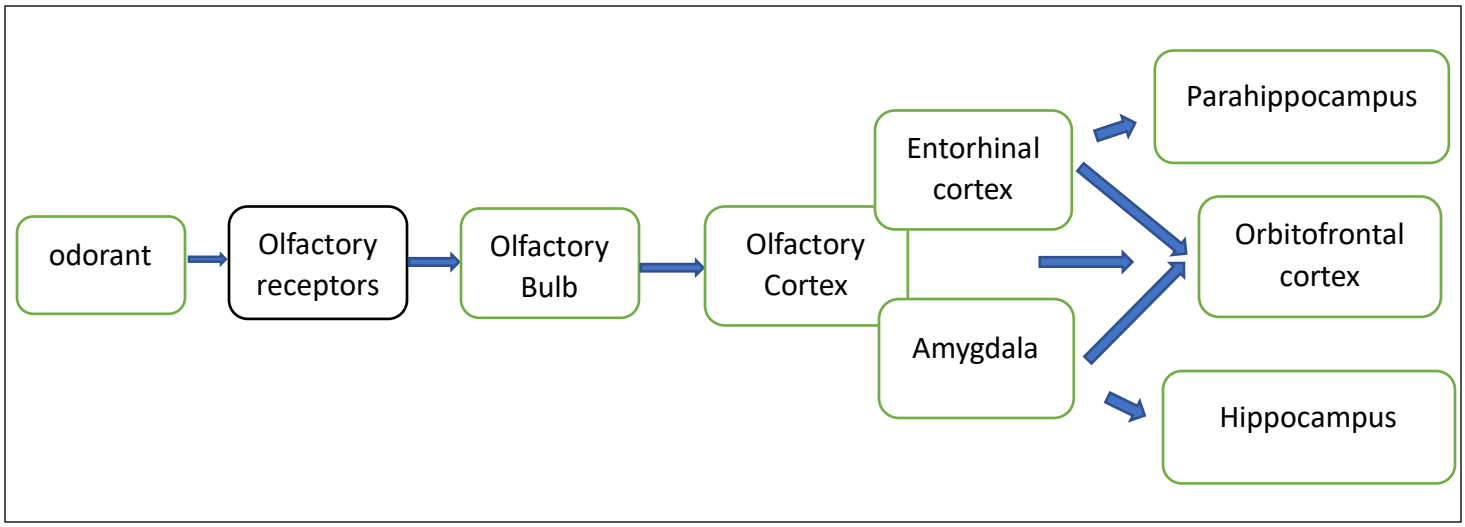


Figure 2.2.1 Brain regions involved in olfactory processes (adapted from (Fjaeldstad et al., 2021; Iizuka et al., 2021; McDonald & Mott, 2017; Stadlbauer et al., 2016)**).**

2.4 Olfactory Dysfunction

Losing the sense of smell may be dangerous for people as it goes often unnoticed. A person may lose their sense of smell, but they may be unaware of it, unlike the sense of sight or hearing, that when lost, people notice any change immediately (Amal et al., 2019). When a person loses their sense of smell, they may be exposed to danger, and this could have serious repercussions for themselves, their community, or their environment. This form of risk can affect anyone who has lost their sense of smell, whether they are men or women, young, middle-aged, elderly, or children. Some people may not realise the dangers that may derive from losing their sense of smell, and it is important for their medical and community support systems to educate them about the dangers of losing their sense of smell. Olfactory abilities might be influenced by many factors such as sex, smoking, lesions caused by respiratory infections, head injury, genetic factors, exposure to some chemicals and usage of certain medications (Ciofalo et al., 2006; Doty, 2017; Mullol et al., 2012). Olfactory dysfunction can have worrisome causes, as the loss of sense of smell is a common symptom of neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease (Attems et al., 2015). At the neurological level, an impaired sense of smell can lead to long-term changes in the structure of the brain. As the areas responsible for the sense of smell, such as the olfactory bulb, shrink, other areas related to the sense of smell also shrink such as the olfactory cortex and other areas in the limbic system. Olfactory deficits can also occur with normal ageing (Rey et al., 2018; Rouby et al., 2011). For example, the ability for

smell identification may start to decline from the 4th decade of life in some people as a result of the normal process of ageing (Zhang & Wang, 2017). This meta-analysis study by Zhang and Wang (2017) indicates that olfactory dysfunction, manifesting in odour identification tasks, starts in the 5th decade of life in healthy participants. However, the study tested olfactory identification only and this limits generalisability of the findings.

However, it is also the case that the areas of the brain that shrink as a consequence of loss of sense of smell can grow again if the sense of smell is restored (Kollndorfer et al., 2014). This can occur either following treatment with medication or following olfactory therapy as shown by Kollndorfer et al. (2014) in their study. Using fMRI, they found that after 12 weeks of olfaction training, olfactory training induced alteration in connectivity within functional networks as this training can induce the process of neurons reorganization. In this study, patients had to choose four out of six odorants options (vanilla, rose, cinnamon, orange, banana and menthol) diluted to standard level and had to expose themselves to these odours twice a day and take one deep sniff of every odour. However, the sample size of this study is very small as seven patients were studied and only one patient had anosmia (total olfactory loss) (Table 2.4.1). When sense of smell is restored via training or other ways, the olfactory bulb, olfactory cortex and other areas in the limbic system all regain volume (Huart et al., 2013). Importantly, anosmia is a total inability to perceive odour sensations while microsmia is a condition in which an individual experiences a decrease in their ability to perceive smell (Doty, 1995).

2.4.1 The common cause of Anosmia.

Cause of Anosmia	Possible recovery time with/without medical attention
Sinonasal diseases	Up to 8 weeks
Infectious diseases	Up to 8 weeks
Toxic exposure	Up to several months after stop of exposure to a toxic agent
Head trauma	About 2 months
Neurodegenerative disorders	Approximately 12 weeks
Idiopathic	Unknown

Adapted from (Babaei et al., 2021; Boesveldt et al., 2017; Gobba & Abbacchini, 2012; Kollndorfer et al., 2014; Mueller & Hummel, 2009; Nordin & Brämerson, 2008; Rashid et al., 2021).

2.4.1 Causes of olfactory dysfunction

Across their lifetime, individuals may experience loss of the sense of smell due to different reasons. Some of these reasons are due to factors that are out of an individual's control, others are due to factors related to the choices made by individuals. Olfactory dysfunction may occur as a consequence of head trauma, respiratory infection, neurodegenerative disorders, and exposure to toxic chemicals, but also following usage of certain drugs, pollution and smoking that all can cause deficits in the olfactory system (Ciofalo et al., 2006; Mullol et al., 2012). Individually, everyone is in danger of exposure to such causes of olfactory impairment.

Virus

Viral infection can damage the olfactory system by affecting the olfactory neuroepithelium that is sensitive to flu infection leading to the individual affected to lose their sense of smell (Doty, 2009). Recently, support for the notion that viral infection can

target the olfactory system has emerged from evidence that the COVID-19 virus, which has caused a global pandemic, causes amongst its earliest symptoms loss of sense of smell (Lechien et al., 2020; Ugurlu et al., 2021). In fact, loss of sense of smell and taste has become a strong indicator of having contracted a COVID-19 infection and it is used as an early symptom indicating that an individual might have contracted this viral infection in addition to other clinical symptoms (Menni et al., 2020).

Smoking

Smoking is also considered a risk factor for olfactory dysfunction (Ajmani et al., 2017). However, olfactory function can be regained by individuals after quitting smoking (Dinc et al., 2020). This restoration process, however, takes time and the time required to return to normal or close to normal depends on the extent of damage, how much time has elapsed since ceasing smoking and, in the case of other causes, the nature of the cause of the impairment of olfactory function (Siegel et al., 2019). For example, in the case of squamous metaplasia of the olfactory mucosa, this condition needs approximately 6 months to be resolved after quitting smoking, while damage caused by inflammation [measured by C-reactive protein (CRP) levels] takes up to 5 years to recover (Hastie et al., 2008; J. S. Lee et al., 1994). In the case of olfactory dysfunction caused by a vascular cause consequent to smoking, it might take from 15 to 20 years for the olfactory function to be restored to the same level of olfactory functioning as that of people who have never smoked. This level of olfactory decline is considered a good predictor of development of cardiovascular issues and has also shown modest evidence of predicting incidence of

heart disease (Conen et al., 2011; Siegel et al., 2019). The olfactory epithelium has the capacity to regenerate in approximately up to 90 days. This regeneration capacity might provide a reasonable explanation for why there is a significant decrease in olfactory function in smokers but not in former smokers or in people who have never smoked (Ajmani et al., 2017; Graziadei et al., 1980; Murphy, 2002). Other possible reasons affecting restoration of olfactory function, including cumulative pack-year smoking (i.e. number of years of smoking), number of cigarettes per day and duration of smoking, must also be taken into account (Ajmani et al., 2017; Dinc et al., 2020). Dinc et al (2020) carried out a cross-sectional study with 28 adult volunteers and measured their olfactory ability immediately before they stopped smoking and 45 days after they had stopped. They also took into account duration of smoking and number of cigarettes per day and found significant olfactory improvement after quitting smoking. However, they found a negative association between duration of smoking and olfactory function, indicating that longer smoking durations may affect the extent of olfactory improvement after stopping (Dinc et al., 2020).

Head trauma

In the case of head trauma causing olfactory dysfunction, recovery may occur relatively soon after the trauma or gradually within two years with regeneration of the relevant nerve fibres. Their reconnection with the olfactory bulb, however, depends on the severity of the trauma itself (Mueller & Hummel, 2009). A study indicated that there is a reduction in global neural volume in thirteen of 22 subcortical regions with a 15% smaller

volume in the amygdala being the highest reduction observed, while in the hippocampus a 10% smaller volume was observed in 25 patients with axonal injury compared with the size of these regions of 22 neurologically healthy age and sex matched controls (Warner et al., 2010).

Chemical exposure

As for the effects of chemical exposure or air pollution, a longitudinal study found an association between anosmia and levels of environmental cumulative exposure to particulate matter ($PM_{2.5}$) (Zhang et al., 2021). In this study that included 2690 individuals, the majority of whom were women, these authors found a dose-dependent association between anosmia and increase in $PM_{2.5}$ concentration. The exposure to toxic chemicals may lead to a highly dangerous set of circumstances for olfaction and for the whole body in general (Bessac & Jordt, 2010). Again, the severity of olfactory loss due to chemical or pollution exposure is dependent on the duration of the exposure. There also are several drugs the use of which can impair olfactory function and these include, for example, Tetracyclines (Doxycycline) or chemotherapeutic agents (Bernhardson et al., 2008; Griffin et al., 2010; Thiermann & Buchbauer, 2017). Interestingly, some medication components that are used to treat olfactory deficits can also be the cause of olfaction deterioration and this is the case, for example, for zinc that is used to treat common cold or nasal blockage (Thiermann & Buchbauer, 2017).

Neurodegenerative disorders

Neurodegenerative disorders can cause olfactory dysfunction. Some studies consider olfactory dysfunction as the first symptom (or earliest) of a neurodegenerative disease such as AD or PD (Doty, 2009; Kollndorfer et al., 2014). However, olfactory deficits vary depending on the type of the neurodegenerative disorder. Per example, olfactory dysfunction has been found to be severe in AD and PD while in HD, schizophrenia and multi-infarct dementia the deficit is moderate (Godoy et al., 2014). Moreover, Progressive supranuclear palsy disorder shows minor olfactory deficit (Doty, 2009). The olfactory deficit domain might depend on the type of neurodegenerative disorder. Huntington's disease has been found to be associated with olfactory identification, detection and memory while AD affects early stage olfactory processes such as olfactory identification and discrimination (Doty, 2009; Gilbert et al., 2004). Moreover, Rahayel et at. (2012) suggested that olfactory detection threshold deficits are observed in PD (Rahayel et al., 2012).

Olfactory function can be affected by biological, environmental, pathological and/or incidental effects, factors that all may lead to a reduction in smelling efficiency (Doty, 2017). Molecular genetic risk factors may also play a role in olfactory dysfunction. The apolipoprotein E epsilon 4 allele (*APOE* ε4) is well recognised as a risk factor associated with AD, and the presence of this genotype has been shown to be associated with olfactory dysfunction (Bacon et al., 1998; Oleson & Murphy, 2015). Another genetic risk factor that may alter olfactory function and/or cognition levels is Brain Derived

Neurotrophic Factor (BDNF). This growth factor regulates the proliferation and the survival of neurons' olfactory receptors and is highly expressed in the brain. Studies have shown that brain BDNF expression levels decrease with ageing (Hedner et al., 2010; Simpson et al., 2002). BDNF expression levels are linked to cognition decline and increasing BDNF expression may reduce pathological signs of AD pathogenesis (Buchman et al., 2016).

In summary, the evidence reviewed above indicates that people should be aware of the many conditions that can negatively affect their sense of smell and its neural substrate and, if possible, avoid those circumstances or habits that might lead to damage, since olfactory dysfunction can potentially put an individual's life in danger and pose risks for their surrounding communities. It is very important that individuals are aware of any sense alteration, especially olfactory function, as it can potentially be the cause of close or distant danger. Olfactory function can be weakened gradually and in stages until it is completely lost or gradually returns to its normal functions when no permanent damage has occurred. Thus, it is important to investigate, characterise and quantify deterioration in the sense of smell and treat it in a prompt and appropriate way to prevent its permanent loss.

2.4.2 Olfactory dysfunction in Ageing

In normal ageing, there is a decline in cognition and olfactory function. Decrease in function in some of the olfactory domains has also been shown to increase the risk of developing dementia (Karunanayaka et al., 2017; Miller et al., 2008; Wang et al., 2010).

Indeed, there are suggestions in the literature that ageing is the strongest risk factor for olfactory dysfunction even more than smoking (Doty, 2009; Doty et al., 1984). There is an association between age and olfactory memory in the elderly as increasing age has been found associated with poorer olfactory memory (Larsson et al., 2016). These authors included 2280 participants in their study, aged 60-100 years old, and measured olfactory memory, specifically odour recognition and odour identification, using the same 16 odour "Sniffin" test (Croy et al., 2015). In this test, after presentation of the stimulus odours, in the odour recognition part of the test, the participants had to decide if the odour were old or new and then they were asked to give a verbal description of the odour. If they failed, they were provided with four written responses from which to choose the best match. However, although time consuming and the long duration of the test which may affect odorant memory retrieval, the test was not sensitive enough and did not classify the degree of olfactory deficit. A study has also shown a significant association between olfactory impairment and ageing (Brämerson et al., 2004). In this study, Brämerson et al, (2004) investigated odour identification in 1387 participants using the Scandinavian Odour Identification Test (Nordin et al., 1998) that includes 16 odorants only. The findings showed that 19.1% of the participants had olfactory dysfunction identifying 13.3% with hyposmia and 5.8% with anosmia, showing a significant association between olfactory deficits and ageing and sex (male participants had worse performance). There is also evidence of an age-related negative relationship in the deterioration of the sense of smell and this can be found in all aspects of olfactory function, including smell identification, smell detection and smell recognition (Mullol et al., 2012). Mullol et al, (2012) ran a cross-

sectional study that took the form of a survey to evaluate olfactory function among more than 9000 general population newspaper readers of varying age (age range 5 to 91 years old). However, the findings of this study are limited by the testing of odour detection, odour memory (recognition) and odour identification (the ability to identify odorants) using only four odorants. Not every elderly would definitely have olfactory deficits but the evidence suggests that it is a common finding (Murphy et al., 2002). In their study, Murphy et al, (2002) investigated olfactory function in 2491 participants aged 53 to 97 years and concluded that olfactory impairment is common among older adults and increases with age. The age point that represents the greatest risk for possible smell loss is not certain; some research has indicated that smell identification ability starts to be altered after the third/fourth decade of life, while other studies have suggested that decline starts in the fifth/sixth decade, with other even suggesting that it might be a much later occurrence with a start in the seventh decade of life (Brämerson et al., 2004; Doty et al., 1984; Seow et al., 2016; Zhang & Wang, 2017). An fMRI study of normal healthy elderly showed that decline can be detected before the age of 65 years (Wang et al., 2016). This fMRI study was done on 43 health volunteers whose olfactory function was investigated using smell identification (i.e. the ability to identify an odorant). A negative association between age and olfactory related activation was found in regions that the authors defined as located in higher-order structures in the central olfactory system, including bilateral dorsolateral prefrontal cortex, left insular cortex and left orbitofrontal cortex, but not in the primary olfactory cortex (Wang et al., 2016). However, loss of sense of smell, either partial or complete, can occur at every age and this impairment could flag the presence of some

serious medical condition. Indeed, In neurodegenerative disorders, especially Parkinson's and Alzheimer's disease, approximately 85-90% of cases experience olfactory dysfunction in the earliest stage of the disease (Doty, 2009; Godoy et al., 2014).

Adams and her colleagues (2018) enrolled 2906 participants (age range 57 to 85 years) and in their report they suggested that older adults with normal cognition and olfactory identification difficulties were at higher risk of developing dementia. Indeed, in this study these authors showed that elderly individuals with olfactory dysfunction were twice more likely to develop dementia within 5 years than those who had normal olfaction (Adams et al., 2018). This study however, used a five-item olfaction test [Sniffin Sticks (Lundström et al., 2003)] and participants who identified four or five odours correctly were considered as normal, otherwise participants were classified as having an olfaction deficit. There is also some suggestion that, among the elderly population with olfactory impairments, ageing has a strong association with olfactory decline and this is more so for men than for women (Doty, 2009). It is still not known why olfactory function declines in ageing and it is not clear whether the causes of decline in ageing overlap with the causes of olfactory dysfunction in neurodegenerative disorders or whether there is an interactive effect between the two conditions. Importantly, the typical AD pathology depositions that are found in the olfactory system (such as in the olfactory bulb) in the brain of AD patients are not present in the olfactory system of cognitively healthy elderly controls (Attems et al., 2005). Moreover, in animal lab models such as aged dogs and aged monkeys, AD pathological depositions, such as tau and/or Amyloid-β deposits have not been found (Attems et al., 2005; Hirai et al., 1996). Taken together, the available

evidence suggests, therefore, that decline in olfactory functions occurs for different reasons in ageing and in neurodegeneration.

Olfactory function has also been found to correlate with the volume of the olfactory bulb and cortical regions in healthy people (Tremblay et al., 2020). There is a strong association between olfactory bulb volume and odour threshold sensitivity in healthy control individuals since it has been found that a larger olfactory bulb volume correlates with a higher odour detection sensitivity score (Turetsky, 2000). This finding was confirmed in a subsequent study that found that there is a significant correlation between olfactory bulb volume and olfactory function that is observed independently of age (Buschhüter et al., 2008). Buschhüter and their team studied the olfactory bulb of 125 randomly selected participants aged 19 to 79 years; they measured the volume of the olfactory bulb and correlated it with participants' scores on the olfactory threshold test, odour discrimination test and identification test, determined using the Sniffin sticks test (Hummel et al., 1997). However, this 16 odour discrimination test might be uncomfortable because the subject may visualise the target pen or might be disturbed by the blindfold (sleeping mask applied as blindfold) and the test could be culturally biased unless some of the odorants are changed to adapt them to potential cultural differences in some of the smells used (Langstaff et al., 2021). There are also several studies that measure different olfactory-related brain regions. In their neuroimaging study, Yao et al, (2014) found that people with olfactory loss, either anosmia or hyposmia, had low grey matter volume in the primary and secondary olfactory cortex; the reduction was found in the Piriform Cortex (PC), insular cortex (IC), the anterior cingulate cortex (ACC), the orbitofrontal cortex

(OFC) and parahippocampal cortex (Yao et al., 2014). Another study indicated that participants with anosmia (total olfactory loss) show lower grey matter volume in different areas including the limbic system and smaller areas such as the piriform cortex, insular cortex, hippocampus, parahippocampal gyrus and orbitofrontal cortex and more (Bitter et al., 2010). The same study observed that the longer the duration of olfactory loss the larger the atrophy found in relevant brain regions.

2.4.3 Olfactory Dysfunction in Alzheimer's Disease

Olfactory dysfunction has been repeatedly reported in AD cases (Murphy, 2019; Velayudhan, 2015) and it has been suggested that it might represent a preclinical sign of this disease (Doty, 2009; Wilson et al., 2009; Wilson, et al., 2007). People with Mild Cognitive Impairment **(**MCI) and patients with AD dementia show significantly poorer abilities in olfactory identification when compared with healthy participants (significant differences noted between groups in age, sex and education that indicated that groups were not matched) (Seligman et al., 2013)**.** One hundred seventy two patients with probable AD, 112 MCI patients and 132 healthy control adults (aged 50 to 100 years) were included in this study (Seligman et al., 2013). Odour identification ability was assessed using the Sniffin' Sticks Odour Identification test (Hummel et al., 1997) that involves the presentation of 16 odorants with participants having to identify a specific odorant among four multiple choice alternatives. Participants with Mild Cognitive Impairment, that is said to be a prodromal stage of AD (Petersen et al., 1999), have also been shown to achieve low olfactory identification scores (Bahar-Fuchs et al., 2011; Westervelt et al., 2008). Impairment in olfactory function has been significantly associated

with AD-related neuropathology burden in the brain (Sohrabi et al., 2012) and some studies even suggest that olfactory dysfunction may be an early symptom of impending AD. Doty (2009) stated that olfactory dysfunction has become recognised as one of the earliest preclinical signs of AD and Parkinson's disease (Doty, 2009). Moreover, Olofsson et al, (2016) concluded in their study that the decline in long-term episodic memory in their sample was accompanied by odour identification impairment (Olofsson et al., 2016). When studying the association between olfactory function and cognitive function in the ageing population, clinical studies and observational ones have found a significant association between olfactory dysfunction and cognitive decline (Sohrabi et al., 2012). Sohrabi et al, (2012) indicated in their study that odour discrimination was the best significant predictor of cognitive decline after measuring a threshold of olfaction function, discrimination and identification over a three year follow-up among 308 participants aged 46–86 years. These authors used the Sniffin Sticks battery including 16 odorants. They concluded that age is significantly associated with cognitive function and olfaction; olfactory discrimination impairment was the best predictor of future cognitive decline but not olfactory identification (ability to identify an odorant). This study used three separated Sniffin Sticks, but this type of olfactory measurement could be affected by any bacterial contamination or visual effect if a participant sees the target stick.

In a different study, participants with MCI showed higher performance than AD patients but worse than healthy controls in a test of smell identification (ability to identify odorant) (Westervelt et al., 2008). This study examined odour identification using the 12-item Brief Smell Identification Test. The study included patient with different mild

cognitive impairment (MCI) subtypes: 17 participants with amnestic MCI, 46 patients with amnestic-multiple domain MCI and 25 patients with non amnestic MCI, and compared them with 44 AD patients and 21 healthy controls. Although olfactory function did not differ among MCI subgroups, MCI participants showed lower olfactory function than controls but better than those patients with AD dementia (Westervelt et al., 2008).

Overall, it has been suggested that the effect of age-related decline on olfaction is much smaller than the effect of pathologies that lead to cognitive decline. Therefore, it has been suggested that olfactory deficits are more likely to be the outcome of pathologies that lead to cognitive decline rather than physiological ageing (Sohrabi et al., 2012). Moreover, patients with AD are expected to have olfactory deficits early in the course of the disease (Peters et al., 2003). Importantly, a study found that 36% of the elderly with impaired olfaction were more likely to die earlier than those who had normal olfactory function and that is because olfactory dysfunction is more frequently associated with neurodegenerative diseases (Wilson et al., 2011). It has been reported in fact that olfactory deficits can predict mortality in the ageing population. A longitudinal study carried out in the USA, Pinto et al, (2014), included 3005 older adults aged 57–85 years in whom olfactory function was determined. Over the 5-year-period of observation, it was reported that mortality in people with anosmia (a stage of severe olfactory loss) was four times higher than in people with normosmia (normal olfactory function). These authors indicated that mortality associated with olfactory dysfunction was higher than that associated with heart failure (Pinto et al., 2014).

AD causes atrophy in a variety of different brain regions. It is, therefore, expected that it might affect regions responsible for olfactory function as well, since some of the main brain structures affected by AD early in the course of the disease are well connected and receive projections from the olfactory cortex (Du et al., 2020; Tunnard et al., 2011). The volume of the olfactory bulb reflects how effective an individual's olfactory function is and the volume of this structure decreases in AD patients from an early stage (Buschhüter et al., 2008; Thomann et al., 2009). Thomann et al. (2009) measured the volume of the olfactory bulb and tract only and found reduction in the volume of the olfactory bulb in their sample that included 29 patients with mild cognitive impairment and 27 patients fulfilling criteria for probable AD of mild severity, when these were compared with a control group with no significant between group difference in age, sex and education and the reduction was more pronounced in AD (Thomann et al., 2009). Sex differences in the size of the regions of the olfactory system have also been reported (Oliveira-Pinto et al., 2014). These authors studied the olfactory bulb of 7 males and 11 females that they obtained 8 to 18 hours *post mortem*. Their findings suggested that females have 40-50% more neurons and non-neuronal brain cells in the olfactory bulb than males (Oliveira-Pinto et al., 2014). These differences may have an impact on the effectiveness of olfactory function of females and males and how their function is affected in the event of pathology. There may also be an asymmetry in the way olfactory brain regions in the two brain hemispheres may be affected by AD pathology, since there is evidence that indicates that the left hemisphere is affected by AD neurodegeneration more than the right hemisphere (Donix et al., 2013), especially in the earliest stages of the disease. Although

neuropathological changes in the course of AD development are almost similar in both hemispheres, the left hemispheric changes occur earlier in the course of the disease, are more severe and precede change in the right hemisphere (Thompson et al., 2003). There is, in fact, a body of recent findings supporting asymmetry in spreading of atrophy in the early stages of AD (Thompson et al., 2003). In addition, there is also evidence that carriers of the *APOE* ε4 allele have smaller volume of the left hippocampus than the right hippocampus when compared with non carriers and healthy controls (Pievani et al., 2011). This asymmetry is likely to be also reflected in the degeneration of the olfactory system that is said to occur early in AD (Christen-Zaech et al., 2003). There also is a growing consensus that degeneration of these brain regions most likely begins decades before clinical detection of AD symptoms (Vasavada et al., 2015).

Earlier research had also shown that, in addition to the *APOE* ε4 allele being a high-risk factor for AD, the presence of the *APOE* ε4 allele is also associated with olfactory decline in the elderly. In fact, Bacon et al, (1998) observed decreased odour sensitivity among those people carrying a copy of the *APOE* ε4 allele compared with those without, even if they had significantly higher mental status scores than non carriers (Bacon et al., 1998). Many areas of the brain that are involved in olfactory processing are affected by AD since neurofibrillary tangles and neuritic plaques are found in many regions related to olfaction in the brain of AD patients such as, for example, the olfactory bulb and the anterior olfactory nucleus (Price et al., 1991; Wilcock, 1983). These

biological factors may play a role in the decline of olfactory function such as the accumulation of neurofibrillary tangles within the olfactory system. Neurofibrillary tangle density is one of the pathological factors that have been seen as associated with olfactory function in the old age population (Wilson, et al., 2007). Wilson et al's study (2007) aimed to detect olfactory dysfunction using the 12-items Brief Smell Identification test (Doty et al., 1996) in an elderly sample of participants. The sample also included 77 brain autopsies of those study participants who subsequently died. The findings suggested that smell identification deficits in the elderly is due in part to the presence of neurofibrillary pathology in the central olfactory regions particularly in the entorhinal cortex and hippocampus (Wilson, et al., 2007). There also is additional experimental evidence that olfactory dysfunction in AD is indeed induced by pathological changes in the tau protein and is related to accumulation of neurofibrillary tangles (Bahar-Fuchs et al., 2010; Kovács et al., 2001; Ohm & Braak, 1987). There also is additional evidence from an animal model that in AD, higher processing of the amyloid precursor protein (APP) occurs in the olfactory epithelium (Kim et al., 2018). Higher processing of APP results in the presence of β-Amyloid fragments and increasing of neurotoxicity especially in the presence of the β-Amyloid 56 fragment (Kim et al., 2018). This transgenic mouse model study by Kim et al, (2018) found that APP processing was higher in the olfactory regions, especially in the olfactory epithelium more than in any other region of the brain of healthy control and diseased samples from mice expressing the human amyloid-β precursor protein when compared with age matched animal controls. There also are other lesions that may be

found in the olfactory system in the AD brain with or without tangle accumulation (Struble & Clark, 1992).

Some studies have also indicated that deterioration in odour identification is a particularly well known impairment in AD and can even predict progression from MCI to AD dementia (Gray et al., 2001; Velayudhan, 2015). Moreover, older adults who are carriers of the *APOE* ε4 allele showed significant decline in odour identification over a 4 year time period (Calhoun-Haney & Murphy, 2005). Importantly, Oleson and Murphy (2015) found that deficits in olfactory processing in individuals carrying two copies (homozygous) of the *APOE* ε4 allele and AD were significantly greater than in those who carried only one copy (heterozygous) of the ε4 allele or in non carriers individuals (Oleson & Murphy, 2015). Additionally, carriers of the *APOE* ε4 allele have also been shown to have differences in age-related cortical thickness, brain network activity function and cognitive decline in the absence of AD pathogenesis as well as showing age-related memory decline earlier than non-*APOE* ε4 carriers (Rodriguez et al., 2013).

Moreover, another genetic factor that plays a role in the integrity of the olfactory system is the *BDNF* val66met polymorphism that modulates the intracellular trafficking and the activity-dependent secretion of the brain-derived neurotropic factor (BDNF) protein and importantly the met allele inhibits intracellular trafficking and regulation of the BDNF protein secretion (Egan et al., 2003; Hedner et al., 2010). The BDNF supports

neuron survival and transmission. (Poo, 2001). Evidence suggests that the BDNF is involved in regulating the proliferation and survival of neurons responsible for olfactory reception (Hedner et al., 2010; Simpson et al., 2002). Buchman et al., (2016) also found that BDNF expression levels are linked to cognition decline and might potentially reduce AD pathogenesis as a higher BDNF expression level was found associated with lower decline in cognition in the ageing population. These findings were obtained in a sample of 535 older adults whose cognitive status had been determined and for whom brain autopsy after death had been carried out (Buchman et al., 2016). Importantly, the BDNF val66met variant appears to play a role in the disruption of neurogenesis of the olfactory bulb as there is a lower expression level of BDNF and its receptors that was observed in knock-in mice containing a variant form of BDNF (a valine (Val) to methionine (Met) substitution at position 66 in the BDNF (Val66Met)) model study (Bath et al., 2008). Bath et al's study (2008) identified BDNF as critical for the functioning of the olfactory system as it regulates migration and survival of new-born neurons in the adult brain and this study reported that this genetic mutation leads to an impairment in activity-dependent BDNF secretion.

2.4.4 Measuring olfactory function:

There are different types of tests for measuring olfactory functions. The type of test depends on the olfactory domain that needs to be tested. Some tests are for odour identification, discrimination and memory, cognition or detection threshold. The olfactory identification test requires the participant to name correctly olfactory stimuli with/without

alternative choices, while olfactory discrimination and memory tests require the participant to identify the odour stimuli out of a set of other similar odorants following a brief delay (Rahayel et al., 2012). The olfactory threshold test measures the minimal concentration needed for the participant to detect an odour stimulus while the recognition test requires the participant to identify the odorant presented out of several choices after a certain time delay (Rahayel et al., 2012). A variety of commercial olfactory tests have been widely used for decades (table 2.4.3). The domains of olfactory Identification, discrimination and memory are well established as being affected in the early stage of AD (Fusetti et al., 2010) and there is evidence suggesting that olfactory identification and discrimination require higher working memory with judgment and decision making abilities (Sohrabi et al., 2012) indicating that these domains as best predictors of future cognitive deterioration. Moreover, some studies have shown that participants with MCI and AD have poor olfactory identification abilities(Seligman et al., 2013). Other authors have suggested that the odour identification and recognition domains are those olfactory abilities mostly affected by AD while olfactory threshold is mostly affected by PD (Rahayel et al., 2012).

Table 2.4.2: Different type of olfactory function.

Olfactory test name	Number of items	Olfactory domain
Smell Identification Test (UPSIT) (Doty et al., 1984)	40 items	Identification
Odour Discrimination/Memory Test (Choudhury, 2003)	12 items	Discrimination and memory
Sniffin' Sticks Test (Hummel et al., 1997)	33 items	Threshold, discrimination and identification
Brief Smell Identification Test (Doty et al., 1996)	12 items	Identification
Sniffin' TOM (test of odour memory) (Croy et al., 2015)	16 items	Recognition
Quick Smell Identification Test (Jackman & Doty, 2005)	3 items	Identification
Pocket Smell Test (Duff et al., 2002)	3 items	Identification

2.4.5 Brain imaging:

A range of imaging techniques can be used to measure the brain. Different neuroimaging techniques such as structural MRI or functional MRI or Magnetoencephalography MEG have been used in research to predict ageing and/or diseases including olfactory dysfunction. Each technique has advantages and disadvantages. MRI is the most widely used technique to image the brain due to its greater spatial resolution. In fact, MRI can visualizes deeper cortical regions. T1 weighted image signal intensity might be changed in the brain structure due to the change in the brain tissue composition with age (Xifra-Porxas et al., 2021). MEG has a higher temporal resolution and offers a good combination of spatiotemporal resolution given that signal in the magnetic field propagates with very little attenuation and distortion (Baillet, 2017). This technique is sensitive to the electrochemical current flows in and between brain cells.

fMRI measures the level of blood-oxygen-level-dependent (BOLD) signal change. This technique has been used to predict age and disease related BOLD signal changes when performing a task but this method is limited because the hemodynamic response function is slow which makes the time resolution very poor in relation to when a neural event has occurred but this can be resolved (Smith et al., 2011). Even if the fMRI technique has been developed 20 years later than MEG, the use of fMRI in research studies is eight times higher than MEG (Baillet, 2017). fMRI can detect the overall change in energy consumption between two conditions with millimeter precision, while MEG reveals highly synchronized neural activities with millisecond resolution (Liljeström et al., 2009). Clinically, MRI and fMRI are vastly used and relied on in neurology and psychiatry (Engemann et al., 2020).

It would be more beneficial to combine two or more neuroimaging techniques in a study. For example Xifra-Porxas et al. (2021) demonstrated in their study that combining structural MRI with MEG to obtain functional information would lead to a better prediction of brain ageing (Xifra-Porxas et al., 2021). Another study combined MRI, fMRI and MEG and suggested that MEG and fMRI both substantially improved age-prediction when combined with anatomical MRI (Engemann et al., 2020).

All of the above in this chapter suggests that it is important to investigate olfactory dysfunction in AD as it might reveal early pathological changes. This claim is supported

by a variety of evidence including findings of early atrophy in brain regions of the olfactory system, evidence from *APOE* ε4 carriers who are at increased risk of developing sporadic AD and who also show greater olfactory function decline, and evidence of poorer olfactory function scores in AD patients when compared with healthy controls.

There are several studies of the sense of smell in patients with Alzheimer's disease or the elderly, but there are fewer studies on whether olfactory impairment can predict the decline of cognitive ability in the elderly. Moreover, most studies have investigated a single olfaction domain such as smell identification, but very rarely studies have applied multiple olfaction test measurements including self-report and tests of odour identification and odour discrimination/memory. In research in this field, a lot of studies rely on self-reports using a questionnaire with questions about olfaction abilities and built up their outcomes and findings based on self-ratings of individuals' olfactory abilities.

A very limited number of original research studies have tested the reliability of measurements acquired with an olfaction self-report questionnaire as a measure of olfactory abilities in the elderly population. It is also the case that very rarely studies have looked at olfactory abilities as predictors of cognitive decline, especially of Alzheimer's disease. Importantly, the accuracy of the test/tests used to measure olfaction is crucial when investigating olfactory function, especially in a clinical context or when the measure might be used as a predictor of disease. A large meta-analysis study investigated the prevalence of olfactory dysfunction in the healthy general population and the usefulness of different types of olfactory assessment in the detection of olfactory dysfunction. The study revealed that the prevalence of olfactory dysfunction was greater when using

expanded olfactory identification tests compared with when only brief olfaction tests were used (Desiato et al., 2021). This study that included 175,073 participants (age range from 18 to 101 years) detected a 22.2% prevalence of olfactory dysfunction. The interesting finding was that prevalence of olfactory dysfunction was greater when objective olfactory tests were used than when subjective olfactory assessments, such as olfactory questionnaire tests or olfactory self-reports, were used (Desiato et al., 2021).

There are numerous studies that have investigated brain volume and atrophy in the elderly, as well as in patients with Alzheimer's disease and/or mild cognitive impairment. However, only few studies have focused on volumetric/atrophy assessment of all brain regions that are involved in processing of sense of smell in all age groups from young, middle-aged and elderly, as well as in patients with Alzheimer's disease dementia or in patients with mild cognitive impairment (Fleisher et al., 2008; Scahill et al., 2003; Yu et al., 2019). Moreover, there are many studies that have measured the size of a specific olfactory area in the brain, such as the olfactory bulb or the entorhinal cortex (Devanand et al., 2012; Thomann, et al., 2009), but there are no comprehensive studies of all olfaction-related brain regions. Therefore, there is a gap of knowledge that leads to new investigations needed to establish a comprehensive evaluation of all these olfaction-related regions of the brain to clarify the extent to which all olfaction-related brain regions are affected by ageing–related and disease related alterations. It is very important, however, to illustrate which olfactory-related brain regions are more effected by the physiological process of ageing and which are instead more susceptible to pathological processes as those associated with MCI and AD. This approach should clarify whether

selective brain regional volume decreases occur as a result of deterioration of olfactory abilities decline or whether there are other pathological processes involved in the involution of olfaction-related brain regions. Clarifying the extent of healthy and disease related volume loss in olfaction-related brain regions might have an impact on the clinical application of olfaction deterioration for the early identification of neurodegenerative conditions. These measurements can help in diagnosis, disease monitoring and evaluation of potential treatments.

Chapter 3: Aims and objectives

Background

Neurodegenerative diseases can manifest early with different types of symptoms stemming from different types of brain alterations. Olfactory dysfunction is one of these alterations and can occur as a result of Alzheimer's disease, Huntington's disease, Parkinson's disease, pathologies all causing cognitive decline. An assessment of the brain olfactory regions in the usual diagnostic clinical pathway for ageing might have a potential impact on detection of early signs of neurodegenerative diseases that would otherwise go unnoticed. Understanding the signs and symptoms of cognitive decline due to Alzheimer's disease helps clinicians to predict and follow the disease from its earliest stage to understand its course more fully, potentially modifying its course or mitigating its effects on cognition, having, therefore, more opportunities to reduce the risk of developing dementia in those people who have the disease.

In this thesis, the experimental studies will focus on the assessment of olfactory functions in healthy older adults with no known symptoms of dementia to establish whether self-assessment of olfactory function is a reliable measure to detect olfactory dysfunction in the elderly population (experiment 1). I will also assess this against objective measures of current olfactory function (experiment 2). In a larger study including three cohorts, I will compare the olfactory cortex volume (OCV) and hippocampus and surrounding regions volumes in younger age, middle age and elderly healthy adults to

establish the effects of physiological ageing on these brain regions (experiment 3). An additional study will compare olfactory cortex volume and hippocampus volume in a group of older adults and a group of patients with Alzheimer's disease to establish consistency of findings in olfactory cortex degeneration and whether any asymmetry in regional neuronal loss is observed (experiment 4). In the final experiment I will compare the regional volumes of the olfactory cortex and of regions of the hippocampal complex in groups of older adults and two groups of patients with Alzheimer's disease (AD) who will be either at prodromal stage (i.e. Mild Cognitive Impairment (MCI)) or at the mild stage of Alzheimer Disease dementia (experiment 5).

Aim 1: To test the hypothesis that assessment of olfactory function is a useful tool to detect the early stage of AD and establish whether self-assessment of olfactory function is a reliable measure of actual olfactory dysfunction in older adults

Objectives:

1. To demonstrate whether self-awareness of olfactory functions is a suitable method of assessment of actual olfactory function by comparing self-report measures with objective scores obtained on a quantitative olfactory test (see chapter 4, experiment 1).
2. Quantitative assessment of olfactory function in the ageing population using the University of Pennsylvania Smell Identification Test (UPSIT) (Richard L Doty, 1995) and Odour Discrimination and Memory Test (ODMT) (Doty, R. L., 2003), two

olfactory function tests, to test whether there is a link between olfactory dysfunction and decline in cognition (see chapter 4, experiment 2).

Assessment of olfactory dysfunction has been suggested as a promising marker and critical diagnostic means to detect the early stage of many neurodegenerative disorders (Christen-Zaech et al., 2003; Djordjevic et al., 2008; Doty, 2012). To achieve the first aim of this thesis the second study was designed to determine whether there is an olfactory deficit among the ageing population and if this correlates with cognitive dysfunction. The data were stratified by age, sex and smoking history. The olfactory protocol included a self-report assessment, quantitative testing of olfactory abilities including measures of odour identification, odour memory and odour discrimination and other tests to measure cognitive abilities. Self-reported and quantitative measures of olfactory abilities were used to test the level of individuals' awareness of their own skills.

Aim 2: To test whether volumetric assessments of the olfactory cortex, hippocampus volume and associated cortical areas involved in aspects of odour processing, odour emotional memory and odour recognition can reliably detect abnormal levels of cognitive decline indicative of early stage AD.

Objectives:

1. To assess whether the olfactory cortex and areas associated with odour processing show volumetric loss of differing trajectories as a result of the normal

process of ageing or in association with neurodegeneration of the Alzheimer type (see chapters 4 and 5, experiments 3, 4 and 5).

2. To assess whether there is a correlation between volume of the olfactory cortex and related areas, and cognitive performance (see chapter 4, experiment 3).
3. To assess whether trajectories of volume loss in the olfactory cortex and areas associated in odour processing differ by sex (see chapter 4 and 5, experiments 3 and 5).
4. To assess whether there is an effect of the *APOE* ε4 genotype and whether any effect on volumetric measurement is dose dependent (see chapters 4 and 5, experiments 3 and 5).

Neuroimaging studies have repeatedly shown brain abnormalities in AD with significant reduction in the volume of the hippocampus in AD and MCI when compared with healthy controls (Chetelat & Baron, 2003; Shi et al., 2009). To achieve the second aim of this study we included younger cognitively healthy individuals, middle age cognitively healthy individuals, cognitively healthy older adults, individuals with Mild Cognitive Impairment and patients with mild to moderate probable Alzheimer's disease dementia from the Sheffield Ageing and Dementia Database (SADD). A three dimensional MRI scan of the brain was used to derive volumetric indices of olfactory regions and of other regions involved in odour processing together with scores from cognitive tests. For a subgroup of participants genetic status for the *APOE* ε4 allele was also used.

The findings of this experimental work will clarify which volumetric neural regional loss in olfaction related regions is related to the process of physiological ageing and which is more specific of neurodegeneration of the Alzheimer type and in this latter case in which regions the effect of neurodegeneration is already detectable at the MCI stage. The study will also establish whether degeneration of olfactory related regions is more pronounced in the presence of the *APOE* ε4 allele.

Chapter 4: Olfactory dysfunction and cognition decline in ageing population

4.1 Experiment 1: Unawareness of olfactory dysfunction in older adult.

4.1.1 Introduction

Deterioration of olfaction is a common phenomenon observed in the older adult population. A number of factors may cause this deficit including infections, ageing and neurodegenerative diseases. From a phylogenetic perspective, olfaction is deemed one of the oldest sensory systems in mammals (Brattoli et al., 2011). Often taken for granted, the sense of smell is of crucial importance. Although olfaction may appear to have less importance in identifying objects or people compared to vision, this sense plays a crucial social and emotional role that affects an individual's taste and food preferences daily (Sarafoleanu et al., 2009). Furthermore, olfaction represents a strong asset when it comes to detecting danger through odours such as gas leaks and/or other toxic fumes, smoke and rotting food. Strong evidence indicates that olfactory dysfunction occurs with ageing (Zhang & Wang, 2017). From this perspective, olfactory dysfunction is a major public health concern and highlights that 1) measures need to be put in place to alleviate these dangers and 2) olfactory therapy should be considered for older adults.

Several factors have been identified that may cause olfactory dysfunction including stroke, viral infections and ageing (Doty, Shaman, Applebaum, et al., 1984; Lee et al., 2014; Wehling, Naess, et al., 2015). Other factors implicated in the loss of olfactory

function include air-flow and mucous composition, structure of the olfactory neuroepithelium and bulb, and olfactory processing in the brain (Alves et al., 2014). The deterioration of smell due to age is often gradual and therefore not noticeable by the affected individual. Many older individuals with microsmia (decreased smell detection ability) are completely unaware of their olfactory dysfunction (Schiffman, 1993; Wehling et al., 2011; White & Kurtz, 2003). In addition, many people suffering from olfactory dysfunction tend to mistake their impairment for a loss of sense of taste rather than for a loss of sense of smell (Doty et al., 1987).

A study carried out in Sweden and Norway, which included healthy participants between the age of 45-79 years, has demonstrated that unawareness of olfactory dysfunction is frequent in older adults who have healthy cognitive abilities established with a comprehensive neurological assessment (Wehling, Lundervold, et al., 2015). These authors demonstrated that 79% of individuals, who claimed to have no olfactory dysfunction, actually had olfactory impairment (57% had anosmia, i.e. complete loss of olfaction). In a similar study, conducted in the United States on adults aged 53 to 79 years, only 20% of participants who suffered from anosmia were aware of having problems with olfaction (Murphy, 2002). The general consensus in the literature, based on the limited studies conducted thus far, supports the hypothesis that older adults are unaware of their olfactory impairment (Landis et al., 2003; Nordin et al., 1995; Wehling et al., 2011). However, there are some studies that have demonstrated that elderly individuals were able to estimate their olfactory function (Rawal et al., 2014, 2015).

There also is substantial evidence in the literature that has shown that olfactory dysfunction is observed in several neurodegenerative diseases, such as Alzheimer disease, Huntington disease, Stroke and Parkinson disease among others (Doty, 2017). Importantly, several studies suggest that olfactory dysfunction may represent an early predictor of future cognitive impairment (Barresi et al., 2012; Devanand et al., 2000; Growdon et al., 2015). Indeed, olfactory impairment is currently seen as one of the top predictors of impending PD and is observed in Mild Cognitive Impairment (MCI), a potential precursor of AD (Liepelt-Scarfone et al., 2013; Picillo et al., 2014). This evidence highlights the importance of establishing robust tests that might reveal a smell disorder in an older individual.

Importantly, unawareness of olfactory problems may lead to malnutrition and depression (Cook et al., 2017; Temmel et al., 2002). In addition, affected individuals will not be able to access their olfactory memories, a factor that may contribute to the development of depression (Zucco & Bollini, 2011). This latter becomes a prominent concern considering that the presence of depression in individuals with MCI more than doubles the risk for progression to AD dementia (Boyle et al., 2006).

Aim and hypothesis of the study

Aims of the study

The aim of this retrospective study was to determine if olfactory self-reporting is a reliable method to determine olfactory function in the older adult population as there is

limited information in the literature on the assessment of this sensory ability. To achieve this aim, an ageing population in the province of Quebec was assessed and their performance on a self-evaluation method was compared with that achieved on a quantitative evaluation of olfaction in this same population. This study evaluated the reliability of the olfaction self-report questionnaire as a measure of olfactory function in older adults and investigated self-awareness of olfactory function among elderly people since there is strong evidence that has demonstrated that olfactory decline occurs with ageing.

Hypothesis of the study

We hypothesised that olfactory function self-report is not an appropriate method of assessment to measure olfactory function and that elderly individuals will show lack of self-awareness of their olfactory dysfunction. Moreover, we hypothesized that olfactory dysfunction would be very common among the elderly population.

4.1.2 Method and materials:

Participants

A total of 93 individuals, 50 females (age range 80-95 years), 43 males (age range 80-93 years) from Sherbrooke, Laval and Montreal Quebec, agreed to participate in the clinical sub-study entitled Olfactory Response and Cognition in Aging study (ORCA) (Fig.4.1.1). The participants were recruited from the large database of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge) in which only healthy and cognitively fit older adults (>67 years of age) were included (Gaudreau et al., 2007). The

NuAge study recruited 1,793 individuals who were followed up longitudinally and assessed annually between 2004-2008.

Recruitment procedures

NuAge applicants filled in self-report forms about their olfactory state each year from 2004-2008 (T1-T4). The questionnaire included the following questions: “Do you have problems with your sense of smell? Such as decreased smell perception or smelling non-appropriate odours. Response options: “No”, “Yes”, “I don’t know” and “If yes, which one? Complete loss of sense of smell, partial loss of sense of smell, smelling non-appropriate odours, or I don’t know”. For the ORCA study, initial calls were made to the previous NuAge participants asking if they would consider being involved in other studies and/or the ORCA sub-study. For those who agreed to be involved, the telephone Mini Mental State Examination (t-MMSE) (Newkirk et al., 2004) was done. In order to include only cognitively healthy older adults, only the ones who scored ≥18 were enrolled into the ORCA sub-study. Letters were then sent out to each individual asking them to confirm if they wanted to take part in the study. The letter included a self-report olfaction questionnaire (identical to the original NuAge self-report) in which they gave information about their current olfactory state (2015) and a consent form. All the participants signed a consent form in their native language. This study received ethical approval by the Research Centre of Aging Ethical Committee (Quebec REB 2015-477). In 2015/2016, 93 of these participants, who now make-up the ORCA sample also completed a second self-report (2016) in the presence of an evaluator and were also assessed at that time with

the University of Pennsylvania Smell Identification Test (UPSIT) (see figure 4.1.1 for a graphical representation of the time-points of assessment).

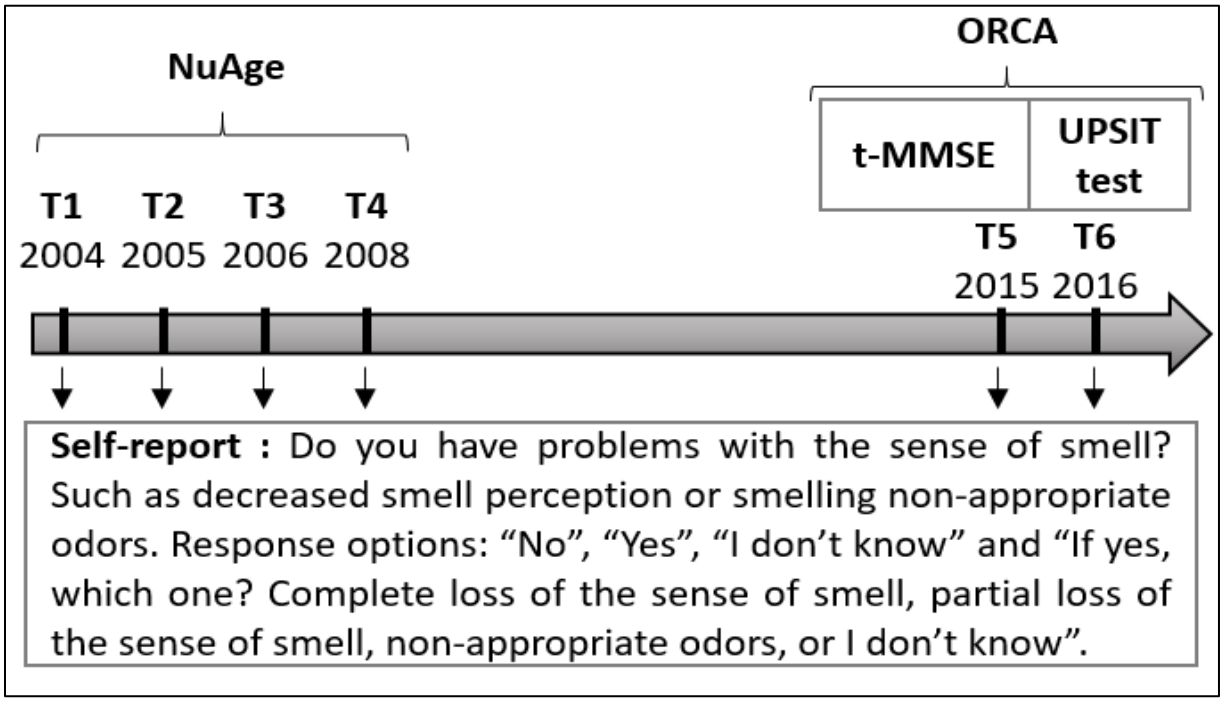


Figure 4.1.1: Experimental design of the ORCA study. The self-report was completed at all time-points (NuAge study: T1- T4, ORCA: 2015 and 2016). The last self-report (2016) was done at the same time as the University of Pennsylvania Smell Identification Test (UPSIT) with an examiner.

The characteristics of the participants are presented in Table 4.1.1. The staff who conducted the interviews were trained and familiar with the olfactory tests used. A $10 compensation was given to each participant to cover incidentals such as parking or public transport costs.

Olfactory evaluation

Participants who agreed to take part in this study were contacted by telephone to arrange for quantitative olfactory testing. The quantitative evaluation used is the current gold-standard test for quantitative assessment of olfaction - the UPSIT. The UPSIT is

commercially available as the Smelling Identification Test (SIT, Sensonics, Inc.) and is the most widely used quantitative olfactory test (Doty, 2017). The UPSIT contains four booklets with a total of 40 different odours. Each smell is sealed in 50 μm of urea-formaldehyde polymer micro-encapsulated attached to a sole binder and positioned on a brown strip. When the brown strip is scraped with a lead pencil, the smell is released. The participant must provide the most appropriate answer between four forced choice alternatives. The test is rated on a maximum score of 40, and determined score ranges correspond to a different olfactory diagnostic category. For women, a score range of 35-40 corresponds to normosmia, of 31-34 to mild microsmia, and of 26-30 to moderate microsmia. For men, a score range of 34-40 corresponds to normosmia, of 30-33 to mild microsmia and of 26-29 to moderate microsmia. For both sexes, a score range of 19-25 corresponds to severe microsmia, of 6-18 to total anosmia and of 0-5 indicates probable malingering.

Statistical analysis

The results of the qualitative and quantitative olfactory tests were first collected by the examiner who carried out the assessment. These results were then re-checked twice by two other examiners. The qualitative results taken in 2015/2016 were compared with those taken between 2004 and 2008 (T1 - T4), and then compared with the quantitative results. Additionally, the results obtained in 2015 were also compared with those obtained in 2016. All statistical analyses were carried out with Graphpad Prism 7 software. Cochran's Q tests were used to compare frequencies of self-report answers in time, and

chi-square tests to compare frequencies of self-report and objective assessment between sexes. Independent sample t test (age, education and raw UPSIT) and Chi square (smoking status) were used to compare the characteristic of men vs. women as shown in Table 4.1.1 The level of significance was set at $p<0.05$. The measure of the sensitivity and specificity of self-report assessment was calculated by comparing the self-report results with the olfactory status evaluated by the UPSIT score.

4.1.3 Results:

The study population had no significant difference in age between males and females while there were differences between males and females in education and smoking as shown in table 4.1.1.

Table 4.1.1: Descriptive characteristics and summary demographics, smoking status and scores on the UPSIT in the study sample.

Variable	Women (Mean ± SD)	Men (Mean ± SD)	p value
Age	85.8 ± 3.8	85.4 ± 3.9	0.58
Education (years)	12.5 ± 4.0	15.7 ± 4.2	0.0004***
Smoking	N	N	0.009**
non smoker	31	15	
Former smoker	19	28	
Current smoker	0	0	
UPSIT	27.6 ± 6.8	25.3 ± 5.1	0.07

SD: standard deviation. N: number of participants.

The results of the 2015/2016 quantitative olfactory tests are shown in (Table 4.1.2) According to the quantitative data obtained in 2015/2016, 94% (87/93) of participants experienced some form of microsmia [mild (24%), moderate (29%) or severe microsmia

(29%) or total anosmia (12%)] (Table 4.1.2, Fig. 4.1.2 A). Even taking into account only the most severe forms of microsmia (moderate, severe microsmia and anosmia) that are those most likely to affect safety and quality of life, the percentage was still quite high (70%, 65/93). Data were also compared between the two sexes. The distribution of olfactory status was not significantly different between men and women in this population (Fig. 4.1.2 B, Chi-square p=0.074). However, the percentage of individuals who suffered from mild microsmia was 18% higher in women, while the percentage of severe microsmia was 24% higher in men.

Table 4.1.2. Participants stratification by qualitative (2015 self-report, done by the participant alone not in front of the examiner) and quantitative (UPSIT) levels of olfaction status expressed in percentage.

OLFACTION DIAGNOSIS	Self-report Answer	UPSIT Women (n=50) Age (80-95)	UPSIT Men (N=43) Age (80-93)	Total (N=93) age (80-95)
Normosmia (%)	No	6 (3/50)	2.3 (1/43)	4.3 (4/93)
	Yes	4 (2/50)	0 (0/43)	2.2 (2/93)
	I don't know	0 (0/50)	0 (0/43)	0 (0/93)
Mild microsmia (%)	No	24 (12/50)	16.2 (7/43)	20.4 (19/93)
	Yes	2 (1/50)	0 (0/43)	1.1 (1/93)
	I don't know	2 (2/50)	0 (0/43)	2.2 (2/93)
Moderate microsmia (%)	No	28 (14/50)	23.3 (10/43)	25.8 (24/93)
	Yes	2 (1/50)	0 (0/43)	1.1 (1/93)
	I don't know	0 (0/50)	4.7 (2/43)	2.2 (2/93)
Severe microsmia (%)	No	12 (6/50)	37.2 (16/43)	23.7 (22/93)
	Yes	4 (2/50)	2.3 (1/43)	3.2 (3/93)
	I don't know	2 (1/50)	2.3 (1/43)	2.2 (2/93)
Anosmia (%)	No	6 (3/50)	7 (3/43)	6.5 (6/93)
	Yes	6 (3/50)	2.3 (1/43)	4.3 (4/93)
	I don't know	0 (0/50)	2.3 (1/43)	1.1 (1/93)
Total (%)		100	100	100

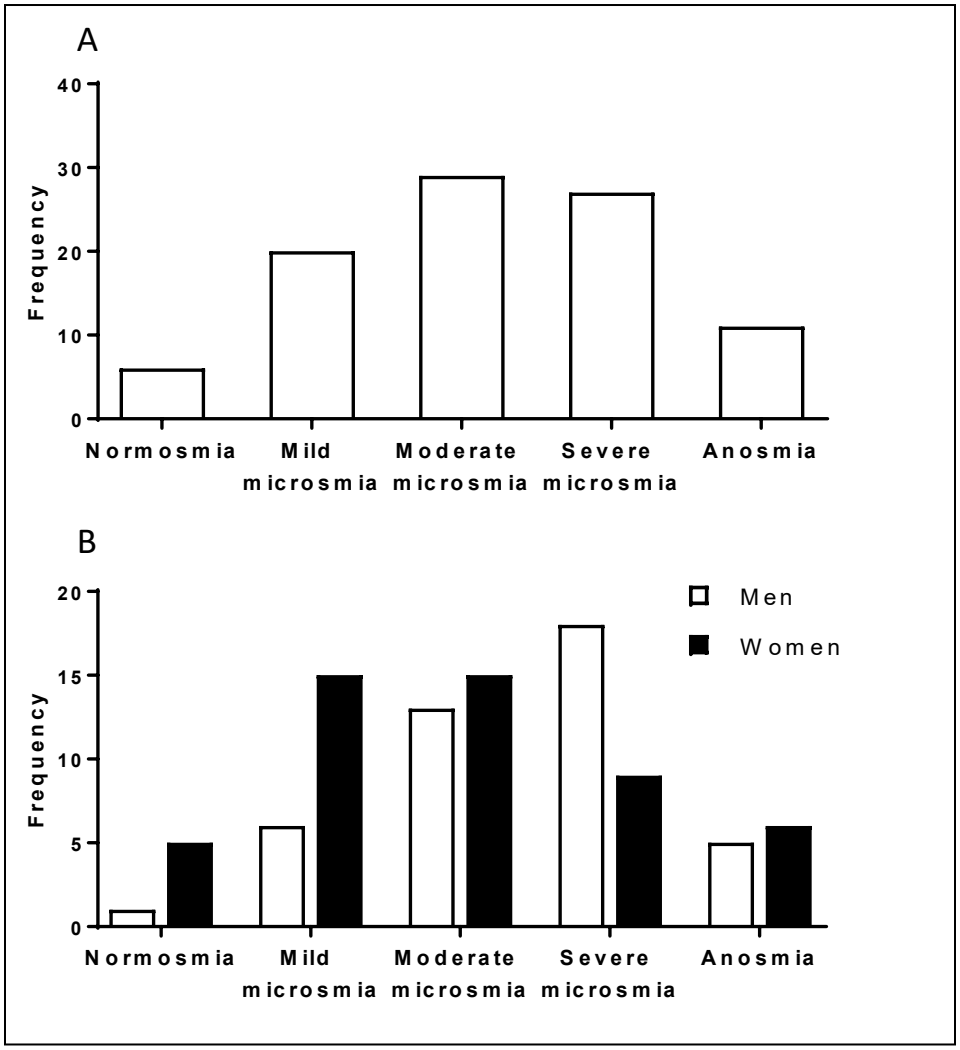


Figure 4.1.2: Frequency of different levels of olfactory dysfunction among older adults. A) 94% (87/93) of participants have some level of microsmia (males and females combined). B) No difference in the distribution of olfactory status is observed between sexes. (UPSIT result).

The quantitative UPSIT olfactory scores were then compared with the responses on the self-report questionnaire. In sharp contrast to the quantitative results, the qualitative self-report of 2015/2016 demonstrates that 81% (75/93) of participants claimed that they did not experience any problems with olfaction (Fig. 4.1.3 A). Within these 75 individuals, 95% (71/75) had some form of microsmia. It is worth mentioning that 91% (10/11) of participants who claimed to have olfactory dysfunction did actually have a form of impairment when assessed with the UPSIT. In addition, 7 out of 93 participants reported that they did not know whether they had any olfactory impairment and 6 of those individuals had microsmia.

We also looked at the distribution of the different olfaction statuses in those participants who claimed not to experience any olfactory dysfunction split by sex (Fig. 4.1.3 B and 4.1.3 C) For females, 76% responded that they had no olfactory impairment, however, 92% (35/38) had deficits as detected by the UPSIT. Specifically, only 8% (3/38) had a normal olfaction status, while 32% (12/38) had mild microsmia, 37% (14/38) had moderate microsmia, 16% (6/38) had severe microsmia and 8% (3/38) had total anosmia (Fig.4.1.3 B, Table 4.1.2). We found a similar situation in the male population, with 86% declaring no deficits when in fact 97% (36/37) did experience problems with smell when tested with the UPSIT. In males, only 3% (1/37) had normal olfactory function, while 19% (7/37) had mild microsmia, 27% (10/37) had moderate microsmia, 43% (16/37) had severe microsmia and 8% (3/37) had total anosmia (Fig. 4.1.3 C, Table 4.1.2). Of note, in the group who claimed that they did not experience any problems with smell, the

difference in the frequency of microsomia between men and women was not significant (p=0.11).

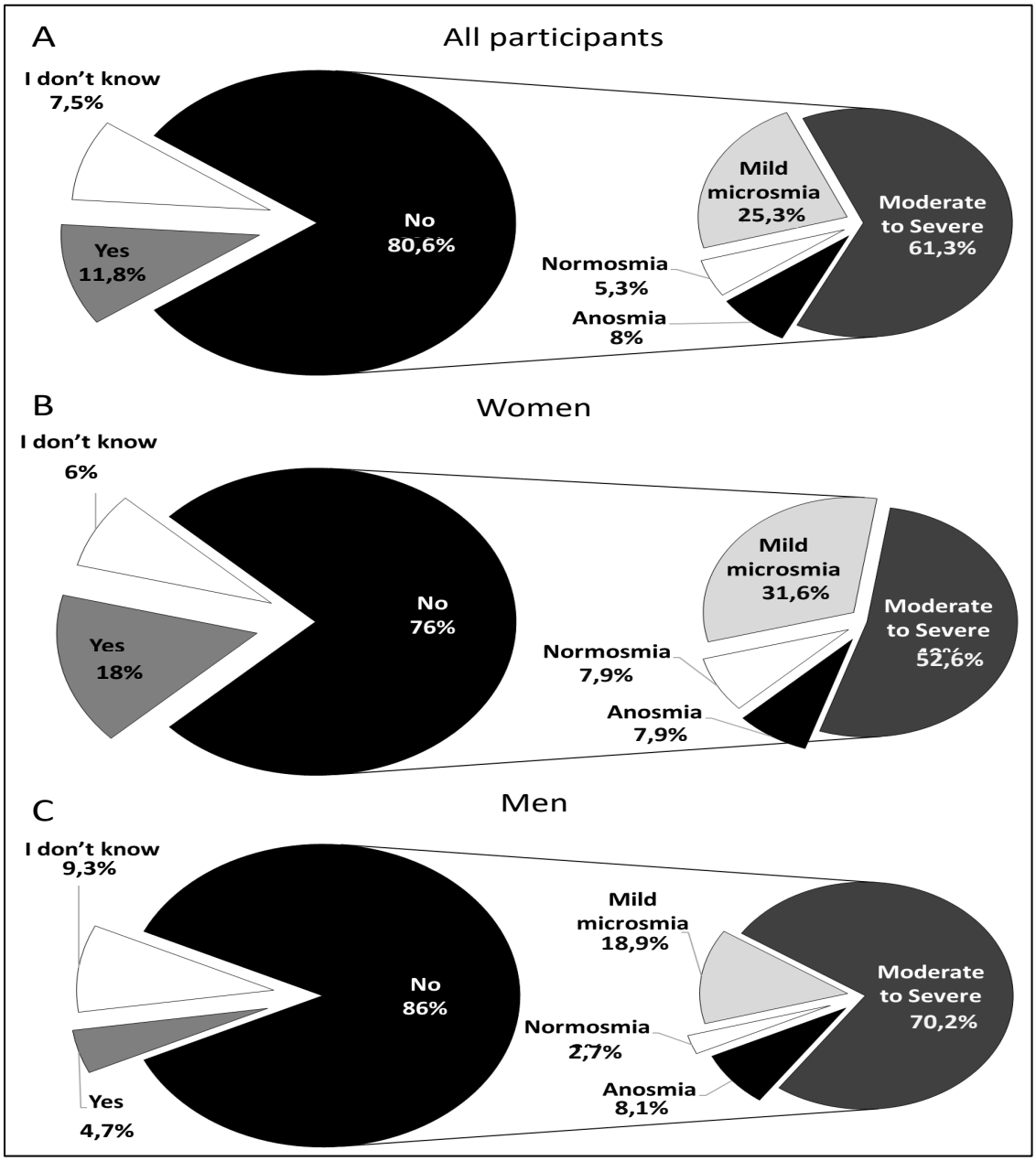


Figure 4.1.3 Unawareness of olfactory dysfunction is not sex related. A) Distribution of the self-report assessment and UPSIT results in all participants (males and females combined) who answered ''No''. B) Distribution of the self-report assessment and UPSIT results in women who answered ''No''. C) Distribution of the self-report assessment and UPSIT results in men who answered ''No''.

These results demonstrate that while the majority of individuals in our sample claimed not to have any problems with smell, they actually had different forms of microsmia. The overall sensitivity of the self-report assessment was 12.3% and the specificity was 80%.

Individuals' self-report assessment of their olfactory function was then evaluated longitudinally across the different assessment time-points, i.e. 2004 to 2008 (T1-T4), and 2015/2016 (Fig. 4.1.4). Fluctuations in the percentages of individuals who reported "no" to the question "do you suffer from any problems in smell" did not change significantly over the years (Cochran's Q test, $p=0.11$). The number of individuals reporting no olfactory dysfunction represented the majority of the participants (81% in 2015). The results of the self-reports done in 2015 and 2016 were also compared to see if the presence of an examiner affected the answer of the participant. Although overall there was no significant variation between the answers in 2015 and 2016 (Cochran's Q test, $p=0.74$) (figure 4.1.5), there were some individual variations of note. Indeed, 5 of the 11 individuals who reported a problem with smell in 2015 changed the answer to "No" when an examiner was present. Interestingly, one of these participants reported a complete loss of smell in 2015. Of note, only 4 of the individuals who reported no problem with sense of smell in 2015 changed their answer to yes in 2016 in the presence of an examiner.

The consistency of the self-report responses was examined by analysing the proportion of individuals who maintained the same response on their olfactory self-report over time. It may be expected that the elderly participants would change their answer

from ‘’no’’ to ‘’ yes’’ to the question “do you suffer from any problems in smell” as their olfaction may decline over time. However, the percentage of participants who answered “No” at T1 and maintained their response significantly dropped overtime (64 participants over 73 maintained their response until T5. Indeed, 88% of the participant who responded “no” at the first time-point (T1) maintained their answer throughout the 5 time-points. In sharp contrast, only 1 out of 8 participants who responded “yes” maintained their answer throughout the years. Although almost all the participants who responded “yes” changed their answer over the years, the variation over time was not significant. This may be explained by the small proportion of individuals who claimed to have an olfactory problem in the self-report assessment (Cochran’s Q test, “no” p=0.001; “yes” p=0.1).

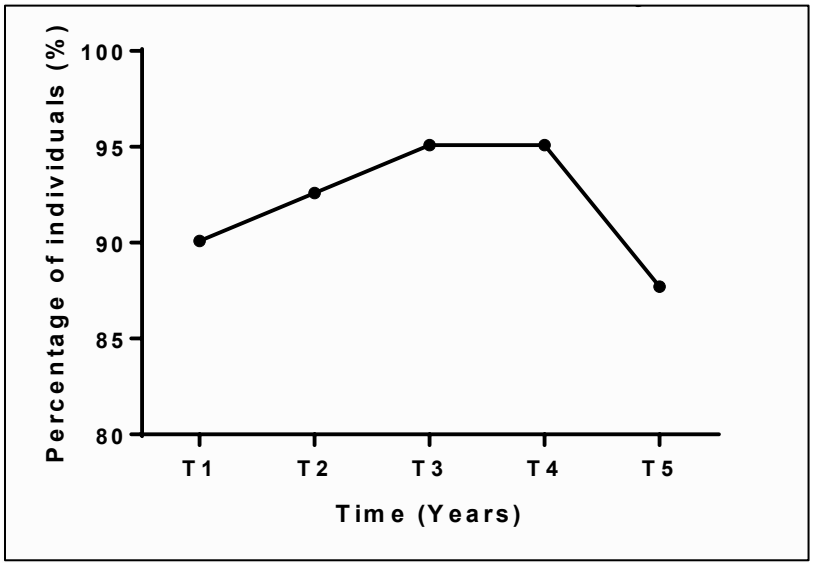


Figure 4.1.4: Percentage of self-reporting rates on the state of olfaction of participants: no change is shown between 2004 and 2016. The percentage of individuals who reported no olfactory deficits remained relatively stable over time. For the purpose of the statistical analysis, the answer ‘I don’t know’ were discarded from the analysis.

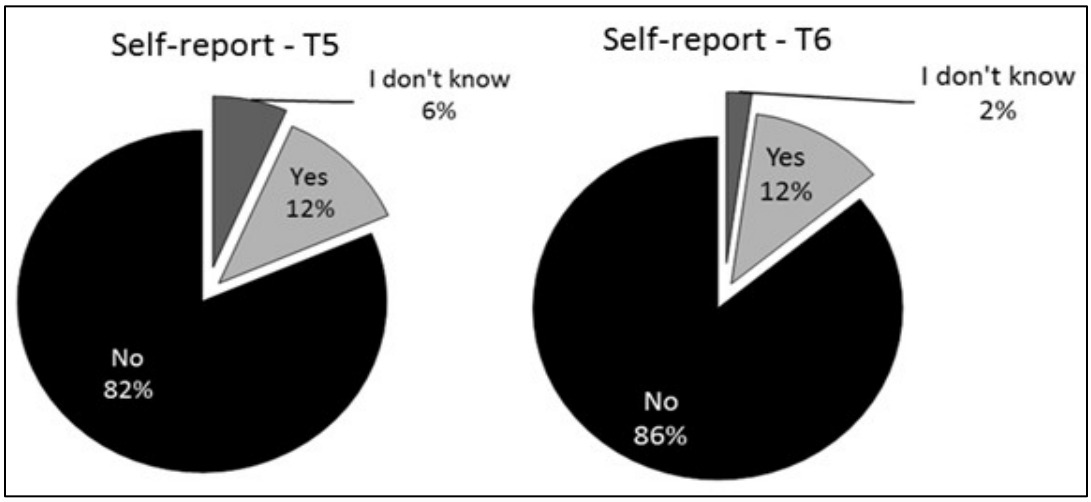


Figure 4.1.5 The Distribution of reporting rates on the state of olfaction of participants: no change when an examiner was present (as shown in T6) or when the examiner was not present (as shown in T5) in response to the self-report.

4.1.4 Discussion

Based on the findings on the quantitative olfactory test (UPSIT), these results demonstrate that 94% (87/93) of the older adult participants in our study had measurable olfactory problems. In contrast, the result of the self-report questionnaire in 2015 (5T) demonstrates that 81% of participants claimed not to suffer from any problems with olfaction. Furthermore, the majority of the participants who appeared to be aware of their olfactory dysfunction in 2004, subsequently changed their answer about the status of their olfactory ability as they became unaware of having an impairment. Additionally, our results show that unawareness of olfactory dysfunction is not sex related. The fact that humans undergo a gradual decline in olfaction with age may make them less aware of their loss over time (Doty, 2017; Krajnik et al., 2015; White & Kurtz, 2003). Indeed, the

objective of this study was to determine whether unawareness of olfactory impairment is a common problem in cognitively well-functioning older adults. Furthermore, the goal was to evaluate the reliability of olfactory self-report questionnaires as a way to determine olfactory capabilities among the elderly in the clinic. These objectives were achieved by comparing a qualitative test of olfaction with a quantitative test (UPSIT) of this function in participants aged 80 to 95 years. Despite the relatively high specificity (correct identification of normosmia) of self-reports (80%), the sensitivity (correct identification of olfactory dysfunction) was very poor (12%) suggesting that self-report questionnaires are not a reliable way to detect olfactory impairment among the older adult population.

Similar to other studies, the results of the present study demonstrate that the majority of elderly individuals have olfactory deficits (Murphy, 2002). However, what is more alarming is that 69% (95% if we include mild microsomia) of the participants who claimed to have no olfactory dysfunction were not aware of their actual olfactory impairment. There is a lack of reports on the association between self-report measures and objective measures of olfaction. This might be due to the fact that the majority of the studies on olfaction in the elderly used a single item self-report (Wehling et al., 2011; Wehling, et al., 2015; White & Kurtz, 2003). However, there are some studies on olfactory awareness that have uses multiple questions self-report (Djordjevic et al., 2008; Rawal et al., 2014; Wehling et al., 2011). Interestingly, it has been shown that, in general, older individuals are not more likely to make errors in the estimation of their faculties than younger individuals. However, they tend to overestimate their olfactory abilities, while the younger population is more likely to underestimate them (White & Kurtz, 2003). This

represents a significant practical danger for older adults as more than 32% of women and 16% of men over the age of 65 years were living alone in 2011 according to statistics in Canada (Canada, 2012) and may be exposed to several risks in everyday life.

It should be noted that from 2004 to 2016, only 10% of the participants switched their answer from no to yes. In the opposite direction, 45% of participants changed their statement from yes to no, a rather significant change. The proportion of the participants who maintained the same answer through the years was also compared. 86% of the participants who answered ‘no’ in other assessments maintained their answer (10% changed their answer to ‘yes’ and 5% to ‘I don’t know’). However, only 27% of the individuals maintained ‘yes’ as an answer throughout the years (45% changed their answer to ‘No’ and 27% to ‘I don’t know’). This may be explained by the fact that unawareness of olfactory dysfunction is common in older people and is not improving with increasing age. The fact that 72% of the participants have disowned their olfactory problems after a few years may seem counter-intuitive. However, this may be due to a deterioration of their olfactory memory since the majority of this specific subgroup of participants (7/11) seemed to suffer from anosmia or a severe form of microsmia. In other words, even though these participants had previously acknowledged their olfactory impairment, subsequently they appeared to have forgotten that they had microsmia. These results suggest that self-reports are a very subjective measure of olfactory abilities and it is neither sensitive nor specific enough to reflect the true olfactory status of an individual.

Olfactory dysfunction has a high prevalence in neurodegenerative diseases such as AD, PD and HD (Alves et al., 2014; Duff et al., 2002), and strong evidence demonstrates that this is an early event in a number of neurological diseases and may be a harbinger of future cognitive impairment (Kjelvik et al., 2014; Vasavada et al., 2015). Based on our results and the results of others, it becomes critical, therefore, to establish quantitative olfactory testing for use in clinical settings for older adults in order to provide education around non-olfactory avoidance of hazardous events (smoke and gas detectors, dating of food and reading food labels, fire-escape plans) and to highlight treatment alternatives for olfactory dysfunction (Cain et al., 1995; Hummel et al., 2009; Pekala et al., 2016; Wysocki et al., 1989) and mental issues related to olfactory loss (depression, loss of appetite). Importantly, quantitative olfactory testing would enable reliable and robust levels of olfactory functions to be determined in the elderly in order to triage which older adult individuals should then go on to have other markers of neurodegenerative diseases assessed. To this date there is no widely adopted policy or clinical algorithm in place for the detection of individuals who have early dementia or indeed may be on the road to cognitive impairment because they may have a neurodegenerative disease. This is despite the fact that there are preventive strategies for dementia-related diseases that have been reliably shown to delay disease progression [healthy eating, exercise (for the body and the mind) and social interactions amongst others] (Barnard et al., 2014; Jimbo et al., 2009; Larson et al., 2006; Radak et al., 2010; Solfrizzi et al., 2011). Significant savings of lives and health-related expenses could be realised should these measures be put into widespread clinical use.

The evidence from the present study suggests that pocket-screening olfactory tests need to become routine in health-care practice. A number of such tests are available including Smell diskettes that can be reused. The UPSIT remains to date the most commonly used test for olfaction. However, there are time constraints with this test. There are several shorter olfaction tests such as Pocket Smell Test (Duff et al., 2002) that are less expensive, and the data would suggest they may be suitable as a first screen (Duff et al., 2002). If the individual misidentifies one of the three odours, then the B-SIT and/or the UPSIT (that contains 40 odours) could be used to investigate the level of microsmia further. In order to consider the best interest of older adults, as well as the financial burden of potential impending neurodegenerative diseases, carrying out olfactory screening may be advisable, using a quantitative olfaction identification test, on individuals >50 years old as part of their annual medical check-up. Unawareness of olfactory deficits may result in a poor and risky quality of life. As an example, if individuals do not realise that they have an olfactory problem, they may find themselves in hazardous situations.

In conclusion, the results of this clinical study suggest that unawareness of olfactory dysfunction is very common in the ageing population and that assessment by a single item self-report (which consists of one question) is not a reliable method for assessing olfactory status in older adults. To date, some studies consider the reliability of self-report questionnaires against quantitative tests still debatable. However, the most findings in the literature still maintain that self-report questionnaires should be used (Landis et al., 2003; Nordin et al., 1995; Wehling et al., 2011). The present study demonstrates that quantitative tests of olfactory function are more reliable, and more

objective, when it comes to evaluating an individual's sense of smell. These results reveal that most older adults are unaware of their olfactory dysfunction and indicate that an olfaction self-report questionnaire is not a valid instrument to assess olfactory function in the ageing population. These findings conclude that there is no correlation between olfactory self-report measures of olfaction and quantitative tests. Thus, it is important to establish quantitative olfactory testing in clinical settings for older adults to provide education around non-olfactory avoidance of hazardous situations and to highlight treatment alternatives for olfaction and mental issues related to olfactory losses (such as depression and loss of appetite). This testing would enable reliable levels of olfactory function to be determined in the elderly in order to identify if an older adult individual should then go on to have other markers assessed for neurodegenerative diseases.

4.2 Experiment 2: Olfactory Dysfunction Associated with Cognitive Decline in ageing.

4.2.1 Introduction:

Neurodegenerative diseases, such as Alzheimer (AD), Parkinson and Huntington disease have a number of symptoms in common including neuropsychiatric deficits, cognitive decline and olfactory dysfunction (Doty, 2012; Gao & Hong, 2008; Gitler et al., 2017). Neurodegenerative processes underlie these symptoms as the neurons lose their ability to function and become structurally damaged (de la Monte & Wands, 2004; Gao & Hong, 2008). With ageing, the prevalence of olfactory dysfunction increases (Zou et al., 2016). However, this is accelerated in neurological disorders.

A main risk factor for a number of neurodegenerative diseases is ageing. This includes conditions such as Alzheimer, Parkinson and Mild Cognitive Impairment (MCI) among others. AD is a chronic disorder that affects millions of individuals worldwide. In many neurological disorders, including AD and MCI, olfactory dysfunction is observed and occurs early in the disease process. Olfactory deficits are a well-validated observation in Mild Cognitive Impairment (MCI) and AD cases (Murphy, 2019; Velayudhan, 2015) and the data suggests this dysfunction is progressive (Westervelt et al., 2008). Studies have also shown that the decline in long-term episodic memory is associated with odour identification impairment (Larsson et al., 2016; Olofsson et al., 2016) and that olfactory deficits often occur prior to cognitive decline (Djordjevic et al.,

2008; Li et al., 2010). Ageing is the biggest risk factor for developing AD (Guerreiro & Bras, 2015). However, the effect of ageing on olfaction is lower than the effect on cognitive decline suggesting that the relationship between olfactory dysfunction and cognitive decline is not mediated by age (Sohrabi et al., 2012). Increased mortality is also observed in individuals with olfactory dysfunction. Indeed, a number of studies have shown that older adults with impaired olfaction are more likely to die than those with normal olfactory function (Doty et al., 1989; Wilson et al., 2011).

Evidence indicates that olfactory dysfunction is one of the earliest preclinical signs of AD (Doty, 2009; Wilson et al., 2009; Wilson, et al., 2007) and that the neuropathological burden in the brain related to AD is associated with olfactory deficits (Doty, 2009; Sohrabi et al., 2012; Wilson, et al., 2007). In early stage AD, neurofibrillary tangles and amyloid accumulation begin to form in the olfactory areas in the brain including the olfactory bulb, amygdala, entorhinal cortex, and orbitofrontal cortex prior to other regions such as the hippocampus (Devanand et al., 2020; Franks et al., 2015; Wesson et al., 2010). Substantial evidence has also shown associations between olfaction dysfunction and atrophy of olfactory brain regions. This includes atrophy of the olfactory bulb and olfactory cortex (Chung et al., 2018; Vasavada et al., 2015). The converse is also true. A number of studies have shown that olfactory bulb volume is associated with odour threshold sensitivity in the healthy population and larger volumes are also associated with higher odour detection sensitivity scores (Buschhüter et al., 2008; Turetsky, 2000).

There is significant evidence in the literature that supports olfactory dysfunction occurs in ageing and that it may be an early sign of a neurodegenerative disease (Attems et al., 2015). However, there are only limited studies in the literature regarding olfactory memory in the ageing population (Choudhury, 2003; Doty et al., 2015; Larsson et al., 2016) and none has been carried out on individuals with >80 years of age. It now becomes important to determine if olfactory memory deficits are observed in older adults at this age and, furthermore, if there are correlations with olfactory function and cognitive scores. In order for clinicians to triage their older adult patients, development of a step-wise protocol to identify pre-clinical MCI/AD cases is essential in order to establish pathways of preventive treatments (exercise, healthy eating, social interactions) that have been shown to help delay the onset of AD (Colcombe et al., 2006; Mendiola-Precoma et al., 2016; Valenzuela et al., 2012; Voss, 2010; Woods et al., 2012).

Aims and hypothesis

Aim of this study

The aims of this study were to determine if deficits in olfactory memory are observed in the older adult population and to determine if olfactory dysfunction correlates with subtle cognitive impairment in the ageing population. This study measured cognition, olfactory function and olfactory memory in older adult individuals with the aim to determine if there were any links between olfactory system function and cognition in older adults who do not have a diagnosis of a neurodegenerative disease.

Hypothesis of the study

In this study, we hypothesised that there will be a significant decrease in the UPSIT and ODMT scores in older adults. There will be a significant correlation between the olfaction scores obtained with either UPSIT and/or ODMT and cognitive scores. In other words, it was predicted that olfactory dysfunction would be associated with lower cognitive functions.

4.2.2 Methods

Participants:

The participants in this study were the same participants as in the previous study (experiment 1). A total of 93 individuals (50 females, 43 males, age range 80-95) from Quebec agreed to participate in the clinical sub-study Olfactory Response and Cognition in Aging (ORCA). A total of 269 letters were sent out and of those who responded (126) 94% agreed to be involved in the study (45% M, 55% F). The NuAge study assessed individuals annually between 2004-2008. For the ORCA study, initial calls were made to the previous NuAge participants asking if they would consider being involved in the ORCA sub-study. For those who agreed, the telephone Mini Mental State Examination (t-MMSE) was done and only cognitively fit adults (≥ 18) were admitted into the ORCA sub-study. The Telephone Interview for Cognitive Status (TICS) (Fong et al., 2009) was also done with the participants. Ethical approval for this study was obtained from the ethics committees of the Research Centre on Aging - CIUSSS de l'Estrie (Quebec REB 2015-

477). Written Informed consent was obtained from the study participants. At the time of the NuAge study, no participants reported any diagnosis of neurological diseases.

Olfactory testing

Each participant who signed the approved consent form was presented with the University of Pennsylvania Smell Identification Test (UPSIT) instruction. The English version of this test consists of 40 four alternative forced choice scratch and sniff item odorants and comes as four envelope sized booklets of 10 items each. Each question has 4 multiple choices as alternative responses and one microencapsulated scratch and sniff odorant. After reading the question and the 4-given choices, the participant has to scratch the given microencapsule located on the right bottom part of the page using a pencil. The odorant will be released after scratching so the participant would identify the sniffed odorant and choose from the 4 alternative response multiple choices the best match for the odorant by circling on the card the perceived correct option of the identified odorant (Doty, 2009; Doty, 2003; Mariano et al., 2018). After each participant completes all questions, a total score is calculated. To identify the type of olfactory function for each participant, the total score of each participant will be compared to that of a normative olfactory function age and sex matched sample using the table provided in the corresponding manual of the test. All odorants in the test correspond to odours usually experienced in a daily life environment. In fact, the UPSIT is a straightforward olfaction function test.

Participants were also tested with the Odour Discrimination and Memory Test (ODMT) (Choudhury, 2003) and followed the procedure provided. The ODMT is designed to measure olfactory memory. This test is used to test the functionality of olfactory memory and the ability to memorise, retain and retrieve a particular odorant after a predetermined set of time intervals. The test comes in one booklet of 12 items, each including 4 multiple forced choice questions. The participant has to scratch the microencapsulated odour using a pencil to release the odorant, sniff and memorise this target odorant and wait for a specific period of time of 10, 30 or 60 seconds. During this waiting time the participant is asked to count backward. After the required waiting time has elapsed, the participant has to scratch 4 microencapsulated odours, then sniff those microencapsulated odours before indicating the best match odorant with the target odorant that the participant has memorised earlier. Four items are used at each of the 3 delay time periods for a total of 12 items with a participant scoring one point for each correctly identified odorant.

Cognitive testing

The t-MMSE and TICS cognitive tests were administered by telephone to all participants. The t-MMSE is a 26-point telephone cognitive screening test that is a modified version of the original AFLI-MMSE (Adult Lifestyle and Function Interview) cognition test (Newkirk et al., 2004). The TICS test measures concentration, short-term memory, sentence repetition, orientation, praxis, and mathematical skills (Fong et al.,

2009). All 93 participants (50 women, 43 men) of the ORCA cohort completed the t-MMSE test. For the TICS cognitive test, 70 participants (38 women, 32 men) completed the test.

Statistical analysis:

The Fisher exact test was used to compare groups, with olfactory function as the categorical variable (categories derived from the UPSIT score). The Wilcoxon test was used to assess for differences in the ODMT between males and females. Pearson correlation was used to indicate if there were a correlation between olfactory function and cognition, education, age and smoking status. We used multiple linear regressions to determine if olfactory dysfunction predicted future cognitive decline in an ageing population. The UPSIT and ODMT were dependent variables and t-MMSE, age, education and smoking status were independent variables. The significance level was set at $p < 0.05$. All statistical analyses were carried out using SPSS software 26 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY) and GraphPad Prism 8 (GraphPad Software San Diego, CA, USA).

4.2.3 Result

Demographics

The descriptive information for this cohort includes age, olfactory and cognition scores, smoking status and education levels for the participants (50 women, 43 men) (Table 4.2.1). There was no significant difference between males and females for age, UPSIT olfactory test scores or cognitive test scores (t-MMSE, TICS). There was a

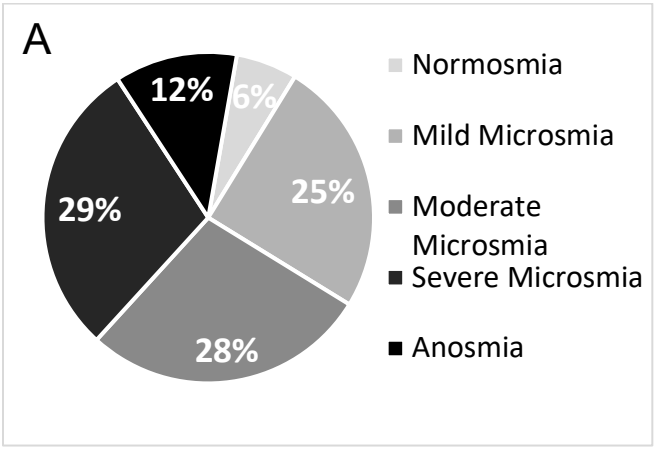
significant difference in the ODMT test (total) scores between males and females (p=0.001), and for years of education (p=0.004). Overall, males had higher education levels than the women in this cohort. There was also a significant difference in the number of smokers between males and females (p=0.013).

Table 4.2.1: Demographics of the ORCA cohort.

	Female (n=50)		Male (n=43)		P value
	Mean	S.D	Mean	S.D	
Age	85.8	3.8	85.4	3.9	0.579
UPSIT	27.6	6.8	25.3	5.1	0.678
ODMT	5.7	2.5	4.2	1.8	0.001**
t-MMSE	23.4	2.3	23.2	1.7	0.621
TICS[1]	19	5.5	17.2	4	0.110
Education	12.5	4.03	15.7	4.2	0.004***
Former smokers	Yes	No	Yes	No	0.013*
	19	31	28	15	

Olfactory function in older adults

As expected, olfactory dysfunction was observed in the ORCA cohort, in both males and females. Overall, 94% of the participants demonstrated some level of olfactory dysfunction with the majority exhibiting moderate to severe microsmia (Fig.4.2.1A). There was no significant difference overall between males and females for the olfactory function subcategories (Fisher exact test, p=0.09). However, there was a significant difference when comparing males and females in mild microsmia (p=0.007). Although 19% of males experienced severe microsmia compared with 10% of females, this difference in proportion was not significant. Between sexes, there was a significant difference in the ODMT total score (Fig.4.2.1B p=0.0012), and at the different time delay intervals (Fig.4.2.1C, Wilcoxon, 10s, z=-1,945, p=0.052; 30s, z=-2.176, p=0.03; 60s, z=-2.849, p=0.004, per 10 sec, 30 sec and 60 sec respectively). Based on previously published olfactory scores in healthy vs older individuals, our results show that this cohort of older adults has deficits in olfactory memory (Doty & Kamath, 2014). In general, females performed better on the ODMT compared to males (p=0.001) (figure 4.2.1 B).



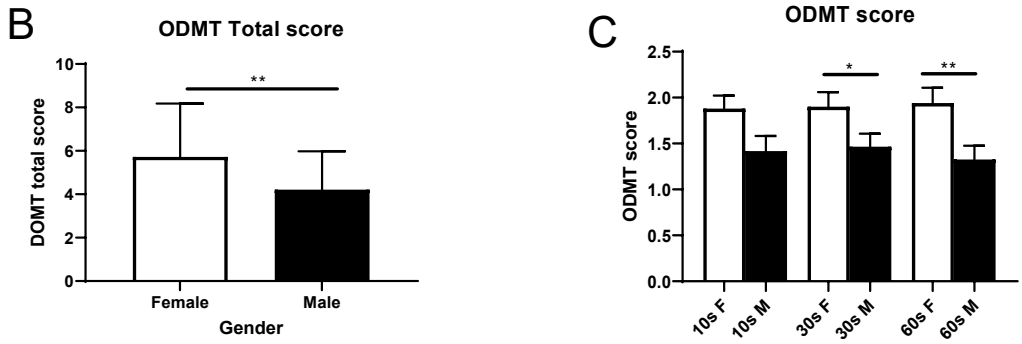


Figure 4.2.1: Deficits in olfactory function in the elderly. A) The pie chart demonstrates the percentage of the ORCA cohort (males and females combined) within each category of olfactory function. Normosmia is normal olfactory function and anosmia is complete loss. B) ODMT total scores in women and men. C) ODMT scores in males and females at 10, 30 and 60 seconds.

A corrplot analysis was then carried out to assess if there was a correlation between olfactory function and cognitive test scores. A significant positive correlation was observed between levels of olfactory function with cognitive scores and trend association in olfactory short-term memory scores with cognitive scores in women (Pearson correlation, UPSIT and t-MMSE, r=0.3, p=0.03; ODMT and t-MMSE, r=0.24, p=0.08 DF=(1,48)). Moreover, a negative association was observed between cognition levels and age (age and t-MMSE, r=-0.29, p=0.04). As expected, and observed previously in the

literature, a negative association was observed between age and UPSIT scores (r=-0.3, p=0.04) in female. No significant correlation between olfactory function scores (UPSIT and ODMT) and cognition levels (t-MMSE) was found in the male cohort.

In order to determine if age, smoking status or education influenced the relationship between the UPSIT or ODMT and cognition scores in women, a linear regression analysis was carried out. In the unadjusted model, there was a significant effect of the UPSIT scores on the t-MMSE results as expected based on findings of the correlation analysis (p=0.03, Table 4.2.2). The significant effect did not survive significance level when accounting for the effects of age, education or smoking when running the models with these variables separately. UPSIT scores, however, still remained a significant predictor of cognition scores when accounting for education (p=0.047) or smoking (p=0.03). For olfactory memory, although a trend negative correlation had been found between the ODMT total score and the t-MMSE in women; in the unadjusted model this did not reach significance level (Table 4.2.3). However, when the model was adjusted for smoking status, the ODMT was a significant predictor of scores on the t-MMSE (p=0.01).

Table 4.2.2 Relationship between olfactory function and cognition in elderly.

UPSIT						
Variables	**Female**			**Male**		
	β	**Std Error**	**p value**	**β**	**Std Error**	**p value**
Unadjusted	**(R^2= 0.09, F= 4.8, p= 0.032*)**			**(R^2= 0.002, F= 0.09, p= 0.77)**		
t-MMSE	0.89	0.40	0.032*	-0.14	0.47	0.77
Adjusted for age (R^2= 0.14, F= 3.8, p= 0.29)				**(R^2= 0.004, F= 0.08, p= 0.91)**		
t-MMSE	0.70	0.41	0.9	-0.14	0.48	0.76
Age	-0.40	0.25	0.11	0.06	0.20	0.77
Adjusted for education (R^2= 0.1, F= 2.5, p= 0.09)				**(R^2= 0.005, F= 0.1, p= 0.91)**		
t-MMSE	0.85	0.42	0.047*	-0.12	0.48	0.79
Education	0.09	0.24	0.68	-0.06	0.19	0.74
Adjusted for smoking (R^2= 0.1, F= 2.4, p= 0.1)				**(R^2= 0.03, F= 0.53, p= 0.56)**		
t-MMSE	0.90	0.41	0.034*	-0.25	0.49	0.60
Smoking	-0.19	1.94	0.92	-1.70	1.71	0.32

Table 4.2.3 Relationship between olfactory short-term memory and cognition.

ODMT						
Variables	Female			Male		
	β	**Std Error**	**p value**	**β**	**Std Error**	**p value**
Unadjusted	**(R^2= 0.07, F= 3.1, p= 0.08)**			**(R^2= 0.005, F= 0.21, p= 0.64)**		
t-MMSE	0.26	0.15	0.08	0.08	0.16	0.64
Adjusted for age (R^2= 0.07, F= 1.8, p= 0.17)				**(R^2= 0.05, F= 1.1, p= 0.33)**		
t-MMSE	0.23	0.16	0.15	0.08	0.16	0.61
Age	-0.07	0.1	0.44	-0.1	0.07	0.16
Adjusted for education (R^2= 0.8, F= 2, p= 0.15)				**(R^2= 0.01, F= 0.12, p= 0.88**)		
t-MMSE	0.23	0.15	0.14	0.07	0.17	0.66
Education	0.08	0.09	0.36	0.01	0.07	0.85
Adjusted for smoking (R^2= 0.18, F= 5.1, p= 0.01*)				**(R^2= 0.04, F= 0.84, p= 0.44)**		
t-MMSE	0.31	0.14	0.034*	0.03	0.17	0.86
Smoking	-1.7	0.67	0.01*	-0.71	0.58	0.23

As smoking appears to have effects on the olfactory scores, olfactory sub-categories were compared between males and females who were former smokers or non smokers. There was a significant difference in olfactory function sub-categories between males and females who were former smokers (Fig.4.2.2A, Fisher exact text, p=0.03). The majority of male participants had severe microsmia (39.3%) while, in contrast, the majority of females in the former smoker sub-group demonstrated mild microsmia (36.8%). In males and females who were non smokers, there was no difference in olfactory sub-categories (Fig.4.2.2B).

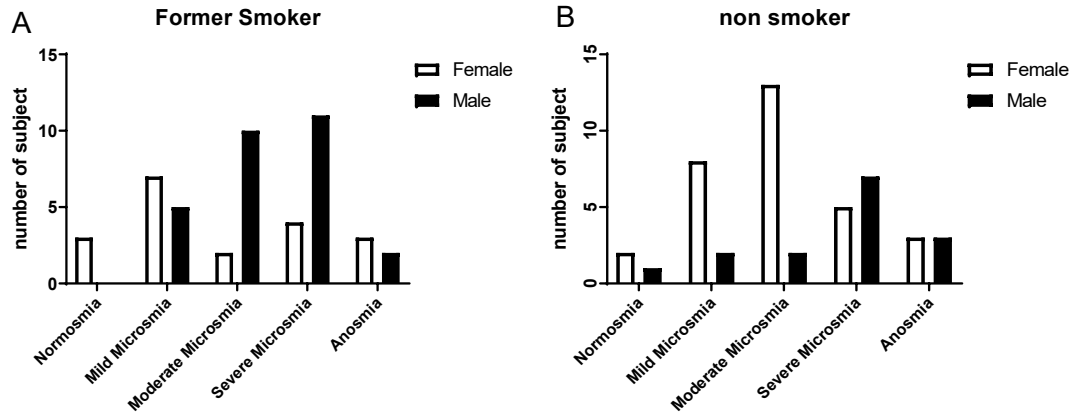


Figure 4.2.2 Olfactory function is influenced by previous history of smoking. A) Distribution of former smoker males and females in olfactory function subcategories. B) Distribution of non smoker women and men in olfactory function subcategories.

Olfactory function sub-categories were also compared between those who never smoked and those who did, in females and males separately. However, there were no significant differences (Fig 4.2.3A Fisher exact text, p=0.116 and B Fisher exact text, p=0.243). In order to determine if age or education affected the relationship between the t-MMSE and the ODMT in women who were former smokers, a regression analysis was carried out. In the unadjusted model, there was a significant effect of the ODMT on the t-MMSE in former smokers (p=0.003). This difference retained statistical significance when the model was adjusted for either age or education, or for both, age and education (Table 4.2.4). In contrast, no such relationship was observed in non smokers.

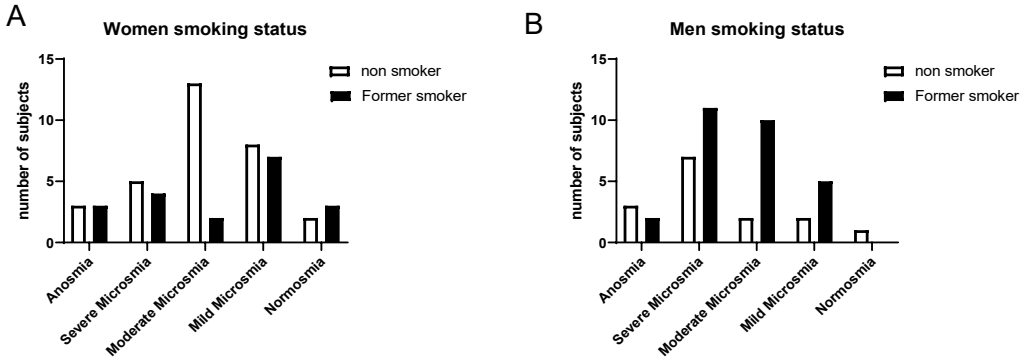


Figure 4.2.3 Olfactory function with the history of smoking. A) Distribution in olfactory function subcategories of former smoker and non smoker women. B) Distribution in olfactory function subcategories of former smoker and non smoker men.

Table 4.2.4 Smoking influences the relationship between olfactory-short memory and cognition levels.

Used to smoke (Female)				Used to smoke (Male)			
Variables	β	Std Error	p value	**Variables**	β	Std Error	P value
Unadjusted (R^2= 0.41, F= 11.9, p= 0.003)**				**Unadjusted (R^2=0.002, F=0.059, p=0.81)**			
t-MMSE	0.786	0.227	0.003**	**t-MMSE**	-0.048	0.198	0.81
Adjusted for the age (R^2= 0.42, F= 5.9, p= 0.01*)				**Adjusted for the age (R^2=0.104, F=1.447, p=0.25)**			
t-MMSE	0.766	0.233	0.005**	**t-MMSE**	-0.009	0.193	0.964
Age	-0.112	0.169	0.518	**Age**	-0.145	0.086	0.105
Adjusted for the education (R^2= 0.52, F= 8.5, p= 0.003)**				**Adjusted for the education (R^2=0.002, F=0.028, p=0.97)**			
t-MMSE	0.728	0.215	0.004**	**t-MMSE**	-0.048	0.205	0.816
Education	0.265	0.143	0.083	**Education**	<0,0001	0.092	0.999
Adjusted for the age and education (R^2= 0.52, F= 5.5, p= 0.009*)				**Adjusted for the age and education (R^2=0.11, F=1.002, p=0.41)**			
t-MMSE	0.713	0.221	0.006**	**t-MMSE**	0.009	0.2	0.963
Age	-0.094	0.16	0.563	**Age**	-0.156	0.091	0.099
Education	0.26	0.147	0.097	**Education**	0.042	0.092	0.657

To determine if other cognitive tests also correlated with the UPSIT and/or ODMT scores, a corrplot analysis was carried out using the TICS scores. Despite the sample size being reduced (n=70) compared to the t-MMSE data (n=93), a significant positive correlation was observed between the UPSIT and TICS scores (r=0.3, p=0.011, DF=69, males and females combined). In order to determine if age, education or smoking

influenced this relationship, a regression analysis was carried out (Table 4.2.5). In the unadjusted model there was a significant positive association (p=0.012, males and females combined). This association still remained significant when adjusting for age (p=0.04), education (p=0.04) or smoking history (p=0.04).

Table 4.2.5 Relationship observed between olfactory function and TICS cognition scores.

UPSIT			
Variables	**β**	**Std Error**	**p value**
Unadjusted (R^2= 0.09, F= 6.7, p= 0.012)			
TICS	0.350	0.135	0.012*
Adjusted for age (R^2= 0.09, F= 3.3, p= 0.041*)			
TICS	0.35	0.14	0.012*
Age	-0.04	0.19	0.82
Adjusted for education (R^2= 0.09, F= 3.3, p= 0.042*)			
TICS	0.35	0.14	0.012*
Education	-0.02	0.16	0.87
Adjusted for smoking (R^2= 0.09, F= 3.4, p= 0.042*)			
TICS	0.35	0.14	0.014*
Smoking	0.21	1.34	0.89

4.2.4 Discussion

Overall, 94% of the ORCA cohort displayed olfactory dysfunction. Deficits in olfactory memory were also present in this cohort. A correlation was observed between olfactory function and both cognitive test scores. Moreover, in women who were former smokers a significant association was found between olfactory short-term memory and cognitive scores. Similar to the findings of the present study, olfactory dysfunction has been reported in the elderly population previously (Murphy, 2002; Wilson, et al., 2007). As individuals age and are in the 6th decade of life and older, their sense of smell deteriorates (Doty et al., 1984; Sinding et al., 2014; Wang et al., 2016). There are no clear causes for this progressive decline in sense of smell. Some have suggested that impairment is caused by a reduction in the olfactory sensory neurons (Rawson et al., 2012), atrophy of olfactory-related brain regions (Doty, 2009; Sohrabi et al., 2012) and/or the influence of genetic variants such as the *APOE* ε4 and/or the BDNFval66met polymorphism (Hedner et al., 2010). This deterioration in olfactory function in the older adult population highlights the potential risk that they might be exposed to or experience dangerous situations. In individuals with olfactory dysfunction, a study revealed that hazardous events were cooking-related incidents (45%), eating spoiled food (25%), and inability to detect gas or smell smoke (23% and 7% respectively) (Santos et al., 2004). Furthermore, a study of older individuals (without dementia) demonstrated that rate of mortality was 36% higher in people with a low score on a quantitative smell test (Wilson et al., 2011). The occurrence of olfactory dysfunction in older adults is very prevalent.

However, unawareness of the impairment is very high in the older age range (Amal et al., 2019; Murphy, 2002).

Anatomically, the olfactory regions in the brain are close to the memory regions (Saive et al., 2014). Olfactory memory has different domains such as discrimination, recognition or sensitivity. The results of the present study show that deficits in olfactory memory are observed in older adults and that males are more impaired than females. This finding is in line with those of other studies that have shown impairments in odour recognition episodic memory in the elderly and that have observed sex effects (Larsson et al., 2016). Odour memory deficits have also been observed in individuals at risk of AD (Schiffman, 2002) as well as in healthy elderly carriers of the *APOE* ε4 allele (Sundermann et al., 2007). The results of the present study demonstrate that the most severe deficits were observed at the 60 seconds time-point. This is in contrast to another study that showed lower ODMT scores at the 10 seconds time-point (Besser et al., 2019). These authors hypothesised that this observation was due to the fact that individuals may remember more odours over longer periods, but that they may show poor initial encoding (Besser et al., 2019). However, the findings of the present study do not lend any support to such a suggestion.

Similar to the findings of the present study, other authors have reported an association between olfactory function and overall cognition in the elderly (Roberts et al., 2016). Indeed some studies have suggested that olfactory dysfunction may predict impending cognitive decline (Devanand et al., 2015; Sohrabi et al., 2012; Wilson, et al.,

2007). Some differences depending on the cognitive test used were observed in the present study. With the t-MMSE, an association with olfactory function was observed only in women. However, with the TICS, this relationship was observed in both males and females. Cognitive tests such as the t-MMSE take less time than the TICS and assess registration of the words to be recalled that will indicate if the participants have hearing difficulties. This is not the case for the TICS and, therefore, the TICS is not suitable for participants with hearing impairments. The other difference between the tests is that educational biases may occur with the t-MMSE and it may show poor sensitivity for detecting MCI (Schmand et al., 1995). Both tests show a correlation with the original form of the MMSE (Fong et al., 2009; Kennedy et al., 2014; Newkirk et al., 2004; Seo et al., 2011). In general, telephone-based cognitive tests examine more memory functioning (Duff et al., 2015). Both tests measure orientation, attention, memory and language. The TICS also measures praxis and mathematical skills, whereas the t-MMSE also includes visual-spatial skills. Overall, however, the discrepancy in findings with these two measures highlights the poor suitability of quick global cognitive screening measures to detect a robust link with olfactory function.

The findings of the present study suggest a link between cognition and olfaction in the women in our cohort. In general, women are more at risk for dementia (Nebel et al., 2018) and are affected by AD more than men (Mazure & Swendsen, 2016), as well as showing more brain atrophy than men (Holland et al., 2013). Furthermore, women have twice the risk of depression, a symptom that increases the risk of developing AD (Nebel et al., 2018). Compared with men, women tend to have a more severe biological

progression of AD (Liesinger et al., 2018; Malpetti et al., 2017) such as more amyloid-β and neurofibrillary tangle deposits (especially in the hippocampus) as well as longer duration of disease (Liesinger et al., 2018).

Smoking is associated with cognitive decline as well as with poorer memory performance (Sabia et al., 2012; Vermeulen et al., 2018). This concurs with the relationship observed in this cohort where an association between olfactory short-term memory and cognition in women who were former smokers was detected. Previous data have shown that smokers are more likely to have olfactory deficits than non-smokers (Katotomichelakis et al., 2007). However, our cohort showed no difference in UPSIT scores between former smokers and those who had never smoked. Possible reasons for this counterintuitive finding might be that the participants of the present study quit smoking >15 years prior to testing (90/93 quit >25 years prior to testing), thus potentially giving the olfactory system time to recover (Ajmani et al., 2017). Inflammation triggered from smoking may resolve within 5 years of being smoking-free, whereas vascular effects normally require up to 15-20 years to restore (Hastie et al., 2008; Siegel et al., 2019).

MCI and AD individuals have olfactory dysfunction, and sex differences have been documented (Roberts et al., 2016; Zou et al., 2016). A possible explanation for the presence of an olfactory deficit in AD patients may be due to the accumulation of neurofibrillary tangles in the olfactory system (Wilson, et al., 2007). In early stage AD, the pathology of neurofibrillary tangles develops in the olfactory bulb (Devanand et al., 2015) and extends to the lateral olfactory regions in the brain (Devanand et al., 2020). The

majority of the literature indicates that smell identification may be the best predictor of cognitive decline. In the present study, the results suggest that olfactory discrimination/memory may also predict impending cognitive decline as an association was observed between olfactory dysfunction and impairment in global cognition. There is evidence to suggest that olfactory identification and discrimination require higher working memory, judgment and decision making capabilities (Sohrabi et al., 2012), functions that support several cognition activities.

This study demonstrates that deficits in the olfactory system are present in older adults and that these are paralleled by lower cognition. Moreover, in elderly women who were former smokers, a significant positive association between olfactory short-term memory and cognitive status was found. These data, and other evidence (Gray et al., 2001), support olfactory testing in older adults. Olfactory screening might be expensive to apply in routine clinical practice. However, some of the olfactory tests are inexpensive. It is important to screen for olfactory impairment not only to detect olfaction dysfunction but potentially to detect deficits in cognitive abilities as some olfactory functions are cognitive-dependent such as episodic odour recognition memory or odour familiarity (Olofsson et al., 2021). It is also important to make the elderly who have olfactory problem aware of their issues and suggest them to do olfactory training, for example, that would be beneficial for their brain in general. There are studies that have suggested that olfactory training leads to increased thickness in olfactory structures and in fronto-temporal areas outside of the olfactory cortex as well as to changes in functional brain activity in a fronto-parietal network associated with higher cognitive abilities (Al Aïn et al., 2019; Fournel et

al., 2017). Our results suggest that olfactory dysfunction may predict impending cognitive decline and highlight the need for olfactory therapy in older adults to improve olfaction and overall well-being. Studying the olfactory system in older adults may identify other markers that may be useful to predict risk of future cognitive decline.

4.3 Experiment 3: Alterations in olfaction-related brain regions in the ageing population: a structural MRI study

4.3.1 Introduction

There are individual differences in the size and composition of the human brain from one person to another, and these differences depend on several factors such as sex, age, education and structural biological factors. These differences may be fundamental and lead to the development of certain diseases or conditions that affect normal life. However, there is individual variation in how different brain regions may be susceptible to disease and how these undergo atrophy (Grajauskas et al., 2019). In other words, not all brain regions would be altered to the same extent by the presence of pathology (Trollor & Valenzuela, 2001). Ageing plays a major role in neurodegenerative disorders such as Alzheimer's disease (Liesinger et al., 2018). Olfactory dysfunction can be an early alteration heralding the presence of a neurodegenerative disorder in ageing. There is, however, evidence of age-related olfactory decline during normal ageing and the central olfactory system is involved in this decline (Wang et al., 2016).

Ageing is associated with reduction in cortical and subcortical grey matter (Jernigan et al., 2001). Studies have repeatedly shown a significant negative association between ageing and brain volume especially grey matter (Courchesne et al., 2000; Raz et al., 2004; Schuff et al., 2012). Raz et al's 2004 study found that grey matter volume increases until it reaches a plateau in the third decade of life. Many aspects may be

involved in causing atrophy in ageing such as a decrease in dendrites and synapses, neuronal death and loss of myelinated nerve fibres (Anderton, 2002; Grajauskas et al., 2019; Pakkenberg, 2003; Peters, 1998). Ageing-related brain atrophy can be the outcome of many different causes that occur in the course of an individual's life span.

Olfactory-related brain regions including the olfactory cortex, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex show greater activation in healthy young adults but less in older adults when engaged in olfactory tasks (Suzuki et al., 2001; Wang et al., 2005). Moreover, elderly participants have lower functional connectivity between these brain regions compared with younger adults (Dennis & Thompson, 2014). Olfactory task-related activation in the brain is greater in the right hemisphere in young adults and that might be due to the predominance of the right side of the brain in olfactory processing (Suzuki et al., 2001). When comparing sexes, women have greater activation in olfactory regions than men (Yousem, et al., 1999). A study has shown acceleration of atrophy in the hippocampus and entorhinal cortex but not in the parahippocampus nor the amygdala with ageing (Jiang et al., 2014). Similarly, another study reported greater volume reduction in the hippocampus, amygdala, orbitofrontal cortex and entorhinal cortex but not in the parahippocampus in healthy ageing (Tamnes et al., 2013). Another study indicates that the volume of the hippocampus and amygdala is stable up to the fourth decade of life and starts its decline in the sixth decade (Smitka et al., 2012). A longitudinal study showed that the highest reduction in volume in all measured brain regions was in the hippocampus, but also reported an increased decline in amygdala volume as well as decline in parahippocampal volume and

a larger reduction in the entorhinal cortex but slightly less reduction in orbitofrontal cortex with age (Sele et al., 2020).

Understanding and measuring volumetric loss in olfactory-related brain regions is important since these are involved in Alzheimer's disease, the most common neurodegenerative disease in ageing, for which olfactory dysfunction and ageing are risk factors. Studying alterations in olfaction-related brain regions might help detection of neurodegenerative diseases at an earlier stage than currently done.

The present study used Magnetic Resonance Imaging to acquire brain scans to determine the extent of volume loss in olfactory-related brain regions during normal ageing. These olfactory-related brain regions include the olfactory cortex, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex. Volumetric measurements were acquired in three age groups, young adults, middle aged adults and older adults, all neurologically healthy to determine the level of regional neural volume loss related to physiological ageing.

Aim and hypothesis:

Aim:

To assess the volume of olfactory cortex and areas associated with odour processing in different age groups in the cognitively healthy population using high resolution 3D structural MRI scans. To test whether normal ageing determines any volumetric alterations of olfactory cortex, hippocampus and other associated areas and

whether there are any differential predictive values of these alterations for neurodegenerative disorders.

Hypothesis:

There would be alteration in olfactory-related brain regions (olfactory cortex, hippocampus, parahippcampus, amygdala, orbitofrontal cortex and entorhinal cortex) in the elderly. The association between age and olfactory-related brain regions would be negative as it is expected that with increasing age there would be greater volume reduction in olfactory-related brain regions. In addition, carriers of the *APOE* ε4 allele, a risk gene for AD, will show greater reductions in volume of olfactory brain regions than *APOE* ε4 allele non-carriers.

4.3.2 Methods and materials

Participants:

Neurologically healthy participants were selected from the cohort of volunteers who had participated as healthy controls in several MRI based research projects coordinated by the Department of Neuroscience at the University of Sheffield between June 2011 and July 2016. These cognitively healthy adult participants were between 20 and 85 years of age. In total, 333 datasets were retrieved and considered for inclusion in this study from the complete University of Sheffield's Department of Neuroscience database that includes neurologically healthy and non-healthy (i.e., with a neurological diagnosis) MRI datasets. Each dataset was inspected with the purpose of identifying

suitable participants and assigning these to three neurologically heathy groups based on the participants' age. The resulting age-based groups were classified as "young", i.e., aged 20-35 (n = 53), "middle-age", i.e., aged 36-65 (n = 66) and "older", i.e., aged 66-85 (n = 93). Clinical profiling via comprehensive neuropsychological testing was available for all participants who were part of the middle-age and older groups (40 years old and older), to confirm their cognitive clinical status as unimpaired. These comprehensive tests include working memory (Digit Span Test – backwards) and short term memory tests (Digit Span Test – forward), verbal long term memory (Prose Memory Test – Immediate) and Verbal learning tests (Paired Associates Learning Test) (De Marco et al., 2017, 2019). This assessment battery also included the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). Eligible participants were those with no neurological deterioration based on different cognitive tests. All eligible participants who had completed a 1.5 T MRI protocol inclusive of a three-dimensional T1-weighted brain image were included. Genetic profiling was also available in a subgroup of these participants and their status for the *APOE* ε4 allele, a well-known risk factor for sporadic AD, was known for 26 participants. There were 9 *APOE* ε4 carriers and 17 *APOE* ε4 non-carriers. Despite the large size of the cohort in the database, not all participants had agreed, however, to provide a biological sample at the time of recruitment. In addition, other demographic variables, i.e. sex and years of education, were available and were included as covariates in the study, given the well-known association found between education and brain regional volume (Mortby et al., 2014).

Written consent was obtained from all participants. Ethical approval and Health Research Authority approval for retrospective data analyses were obtained from the West of Scotland Regional Ethics Committee 5, Reference number: 19/WS/0177. All procedures used to assess participants in this study followed institutional ethical standards and were in compliance with the Helsinki declaration.

MRI acquisition:

Each participant had a three-dimensional T1-weighted scan as part of their MRI protocol. A Turbo Field Echo T1-weighted image sequence was acquired for each participant using a Philips Achieva 1.5 T scanner as part of a single MRI protocol. The T1 image parameters were as follows: voxel size: 1.1 × 1.1 × 0.6 mm (gap 0.6 mm); 250 mm field of view; repetition time 7.4 ms; echo delay time 3.4 ms; matrix size 256 × 256 × 124 and flip angle 8°.

MRI processing:

Pre-processing of MRI images was done using the most updated version of the Statistical Parametric Mapping software package (SPM), i.e., version 12 (Wellcome Centre for Human Neuroimaging, London, UK), running in a Matlab R2016b environment, version 9.1 (Mathworks Inc, Natick, Massachusetts, USA). SPM was used to segment and separate each T1-weighted image into three tissue maps, i.e. grey matter, white matter, and cerebrospinal fluid. Of these, the neural tissue maps (grey matter and white

matter) and the map of cerebrospinal fluid were included in this study in order to address the study hypothesis.

Pre-processing consisted of several steps starting with reorientation. Initially, each T1-weighted anatomical image was displayed on screen to verify whether the participant had any anatomical abnormalities. Once ruled out any abnormality in the target image or any motion artefact that would lead to distorted or unclear signal, the T1-weighted images were manually centred at the anterior commissure and reoriented. As a first step, each nifty image was aligned to the anterior commissure of the brain that was considered as the main anatomical reference landmark. After the reorientation step, images underwent a segmentation process using the "*new segment"* procedure in SPM12 and the overall 3D image of the brain was subdivided into three tissue-classes by separating the intracranial voxels of interest into grey matter, white matter and cerebrospinal fluid (CSF). During the segmentation process, images were segmented in their native space. As a result, modulated warped tissue maps were obtained to be processed in the next step of the preprocessing procedure. Before proceeding to the next step, grey matter segmented images (i.e., files with a "c1" prefix) were quality checked for every individual participant to validate the workflow and identify potential segmentation errors. No segmentation errors were found in the images. The images were then smoothed with an 8-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel to narrow down the variation in individual brains, increase the signal to noise ratio and reduce the impact on voxels. In order to answer the study questions, volumetric grey matter tissue maps were considered for further analysis and were processed applying a regional volume extraction method.

Extraction of Olfactory Region Volumes:

In order to extract and calculate individual total intracranial volumes, tissue-class volumes were individually extracted in SPM12 using the individuals' images containing the segmentation parameter (Malone et al., 2015). Grey matter volume, white matter volume and CSF volume were computed in litres and converted to millilitres to be compatible with the unit of measurement of olfactory brain region volumes during data analysis. Summing the volume of the three sub-maps was carried out to calculate each individual's Total Intracranial Volume.

Regional volumes (in millilitres) were extracted from all segmented, modulated and smoothed grey matter images using the "get_totals" (http://www0.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m) Matlab function to obtain a measurement of the olfactory brain regions of interest. These were defined via the SPM12 toolbox Wake Forest University (WFU) PickAtlas (Maldjian et al., 2003) that was used to specify olfaction-related regions from a human-brain atlas. This was the Automated Anatomical Labelling (AAL) atlas that was used as the reference to create region-of-interest (ROI) masks for each individual region in the MNI space (Tzourio-Mazoyer et al., 2002). The olfactory-related brain regions selected as part of this study were: left/right olfactory cortex, left/right hippocampus, left/right parahippocampus, left/right amygdala, left/right orbitofrontal cortex and left/right entorhinal cortex. However, in addition to the AAL, a number of regions from a second atlas were also included in order to cover the whole set of olfactory regions as thoroughly as possible. To do so, other atlases were

used to identify and create masks for the remaining olfaction-related brain regions. The orbitofrontal cortex mask was taken from the Harvard-Oxford atlas (Desikan et al., 2006) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) and included a combination of medial and lateral orbitofrontal sub-regions in the left and right hemispheres. Moreover, Brodmann areas 28 and 34 (from the Brodmann Areas atlas) were included as 'entorhinal cortex' and respective masks were created. Automatic extraction was done by processing the map of grey matter volume for each ROI for every individual participant using the "get_totals" MATLAB script, separately for both brain hemispheres. The outcome of this method was separate volumetric measures for the left and right ROIs. Summing the ROI volumes from both hemispheres served to obtain the total ROI volume.

Data analysis:

Statistical data analyses were carried out using the SPSS version 26 software (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) and the latest version of GraphPad Prism 9 (GraphPad Software San Diego, CA, USA). Descriptive statistics were run to characterise the study population as shown in table 4.3.1. To explore the data and answer the study question, a one-way ANOVA was run to compare the volume of the selected brain regions of interest among the three groups of neurologically healthy participants in this study. To achieve the aim of the study investigation, analyses of covariance (ANCOVA) were used in the form of Univariate General Linear Model to regress out the influence of education and total intracranial volume (TIV). Shapiro-Wilk

and Kolmogorov-Smirnov test significance levels together with the inspection of the histograms illustrating the frequency distribution of outcome variables were considered as part of the normality diagnostic process to verify if the ROI volumes were normally distributed. Pearson's correlation coefficient was used to investigate the association between age, education and TIV, and each of the volumetric measures. To determine the influence of the *APOE* ε4 allele as a risk factor on the volume of olfactory regions, independent sample t tests were carried out to compare ROI volumes between the small sub-group of *APOE* ε4 carriers and the sub-group of non-carriers. The statistical threshold to define the significance level for the ROI volume comparisons was set at $p < 0.001$.

All contrasts were devised to test the planned experimental hypotheses. The olfactory related brain regions selected in this analysis included the olfactory cortex, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex. The volume of these regions was extracted for the purpose of this study.

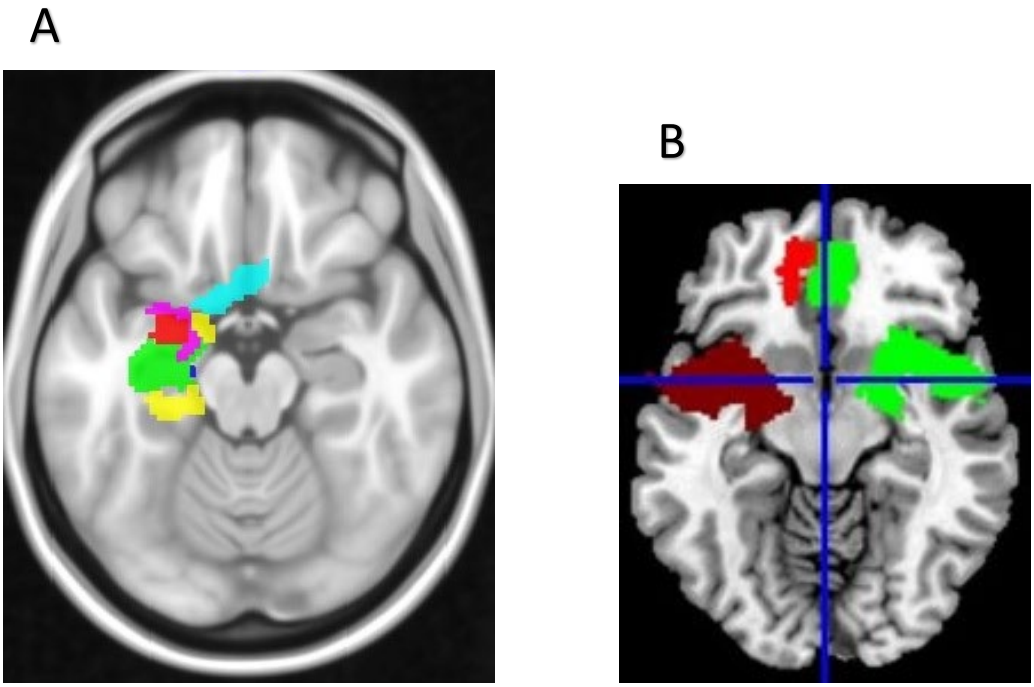


Figure 4.3.1: Volumetric structure of olfactory-related brain regions in the left hemisphere selected as regions of interest: A) Olfactory cortex left (cyan), hippocampus left (light green), parahippcampus left (yellow), Amygdala left (red) and entorhinal cortex left (pink). B) Orbitofrontal cortex left and right in light green and red.

4.3.3 Result

Significant differences in education were found among the three groups (both sexes F=29.2, DF= (2.211), p=0.0001, female F=28.7, DF= (2,126), p=0.0001, and male F=5.04, DF= (2,82), p=0.009). Significant differences were found in all group comparisons in females namely young vs middle age (p=0.003), young vs elderly (p<0.001) and middle age vs elderly (p<0.001). In males significant differences were found only in the young vs elderly (p=0.006) comparison. As expected, significant group differences were found for age.

Table 4.3.1: Demographic Characteristics of the participants

	Female			Male		
		Mean (SD)			Mean (SD)	
	N	Age	Education	N	Age	Education
Young Age	33	25.7(3.8)	16.9(1.9)	20	26.9(4.8)	16.5(3.4)
Middle Age	39	54(8.1)	13.7(5.3)	27	53.4(9.2)	13.8(4.4)
Elderly	57	72.4(4.3)	10.3(4)	38	73.4(4.9)	12.7(4.8)

One-way ANOVA analyses showed significant differences in volume between age groups in all olfaction-related brain regions ($p \leq 0.0001$) (Table 4.3.2), with the lowest volume observed in the oldest sub-group.

Post-hoc multiple comparisons Bonferroni tests showed multiple significant differences among age groups except in the young vs the middle-aged comparison where no significant differences were found for hippocampal and amygdala volumes, both in women and men (Table 4.3.4 and Table 4.3.5). ANCOVA analysis followed by pairwise comparisons of groups showed no significant differences in all olfaction-related brain regions when controlling for education or TIV separately. All ROI volumes were normally distributed except for TIV, white matter and CSF volume. No association was found between age or education and TIV when both sexes were analysed together nor when they were analysed separately (Table 4.3.3).

Table 4.3.2: Region of interest characteristics.

Region of Interest	Atlas	R squared	Below threshold?	P value summary
Olfactory Cortex L	AAL	0.3093	Yes	****
Olfactory Cortex R	AAL	0.3236	Yes	****
Hippocampus L	AAL	0.2456	Yes	****
Hippocampus R	AAL	0.1829	Yes	****
Parahippocampus L	AAL	0.3032	Yes	****
Parahippocampus R	AAL	0.2276	Yes	****
Amygdala L	AAL	0.1512	Yes	****
Amygdala R	AAL	0.1757	Yes	****
Orbitofrontal Cortex L	Harvard-Oxford	0.3941	Yes	****
Orbitofrontal Cortex R	Harvard-Oxford	0.3877	Yes	****
Entorhinal Cortex L	brodmann	0.3688	Yes	****
Entorhinal Cortex R	brodmann	0.2766	Yes	****

Significance level = p <0.001. L: left, R: right, AAL; Automated Anatomical Labelling, Harvard-Oxford: Harvard-Oxford Atlas, Brodmann: Brodmann's areas Atlas, (****): significance level p≤0.0001.

Table 4.3.3: Association of age and education with total intracranial volume TIV (R square and P value are reported).

TIV	Age		Education	
	R^2	p value	R^2	p value
Both sexes	0.0004	0.77	0.0034	0.39
Women	0.008	0.3	0.028	0.055
Men	0.011	0.34	0.037	0.076

Significance level = p <0.05

Olfactory cortex volume:

One-way ANOVA analyses showed significant differences in volume among age groups in OCV (F=49.77, DF=(2,211) p≤0.0001) with a negative association with age. (Figure 4.3.2). *Post-hoc* multiple comparisons Bonferroni tests showed significant differences in regional volumes for all age groups except for the total and left OCV between middle-age and elderly in the male population (Figure 4.3.2, table 4.3.4 for females and table 4.3.5 for males).

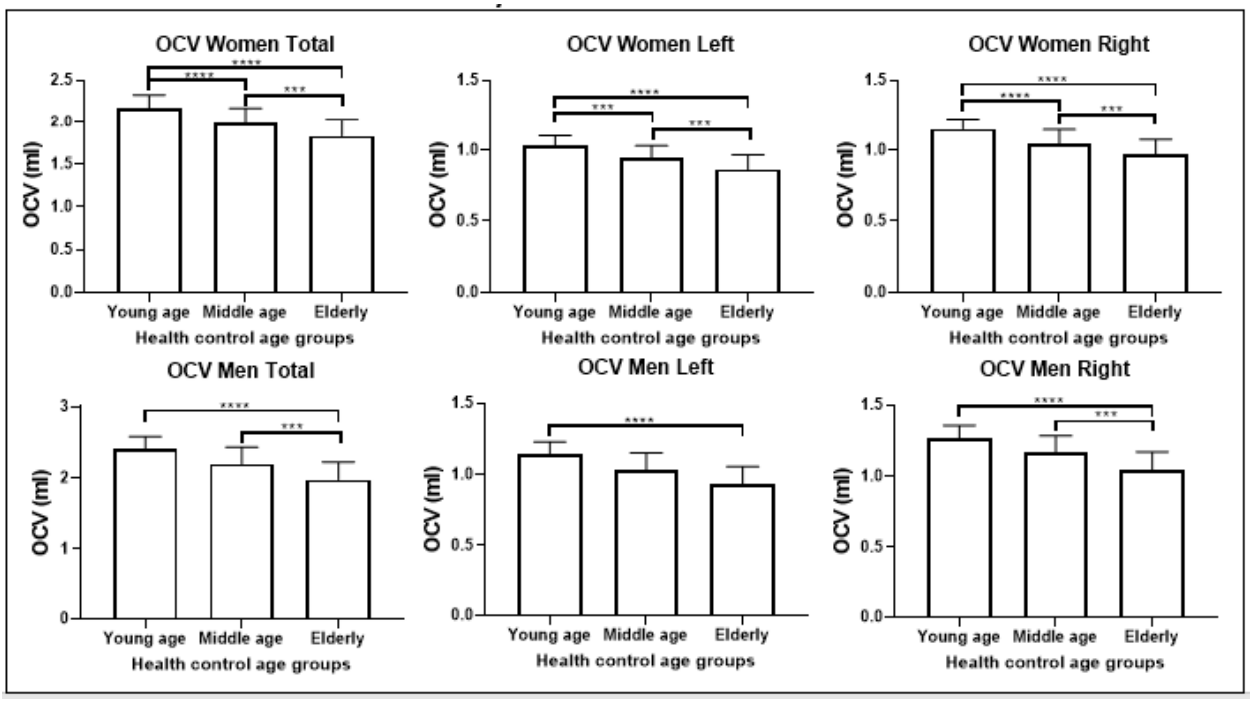


Figure 4.3.2: Olfactory cortex volume differences among groups split by sex and side: comparison of OCV among the three groups (young, middle and elderly) in total, left and right volume for the female population (upper row) and for the male population (lower row).

Table 4.3.4: Significant difference in volume in the olfactory regions observed among female age groups.

	Female	Young vs. Middle Mean Diff. (p Value)	Young vs. Elderly Mean Diff. (p Value)	Middle vs. Elderly Mean Diff. (p Value)			Young vs. Middle Mean Diff. (p Value)	Young vs. Elderly Mean Diff. (p Value)	Middle vs. Elderly Mean Diff. (p Value)
Olfactory Cortex	Total F=37.3, (P=<0.0001)	0.19 (<0.0001)	0.34 (<0.0001)	0.15 (0.0003)	Amygdala	Total F=15.22 (P=<0.0001)	0.08 (0.07)	0.16 (<0.0001)	0.09 (0.007)
	Left F=35.3 (P=<0.0001)	0.09 (0.0001)	0.16 (<0.0001)	0.07 (0.0003)		Left F=13.6 (P=<0.0001)	0.03 (0.08)	0.08 (<0.0001)	0.04 (0.02)
	Right F=37.8 (P=<0.0001)	0.10 (<0.0001)	0.18 (<0.0001)	0.08 (0.0003)		Right F=15.3 (P=<0.0001)	0.04 (0.09)	0.08 (<0.0001)	0.05 (0.006)
Hippocampus	Total F=22.03 (P=<0.0001)	0.23 (0.12)	0.65 (<0.0001)	0.42 (0.0001)	Orbitofrontal cortex	Total F=53.5 (P=<0.0001)	2.15 (<0.0001)	3.91 (<0.0001)	1.76 (<0.0001)
	Left F=21.2 (P=<0.0001)	0.08 (0.43)	0.31 (<0.0001)	0.23 (<0.0001)		Left F=54.6 (P=<0.0001)	1.10 (<0.0001)	1.97 (<0.0001)	0.87 (<0.0001)
	Right F=18.9 (P=<0.0001)	0.14 (0.04)	0.33 (<0.0001)	0.18 (0.002)		Right F=50.4 (P=<0.0001)	1.05 (<0.0001)	1.94 (<0.0001)	0.89 (<0.0001)
Parahippocampus	Total F=34.6 (P=<0.0001)	0.51 (0.0003)	0.96 (<0.0001)	0.46 (0.0002)	Entorhinal Cortex	Total F=35.7 (P=<0.0001)	0.15 (0.002)	0.31 (<0.0001)	0.17 (<0.0001)
	Left F=40.8 (P=<0.0001)	0.25 (<0.0001)	0.48 (<0.0001)	0.23 (<0.0001)		Left F=41.1 (P=<0.0001)	0.08 (0.0005)	0.17 (<0.0001)	0.09 (<0.0001)
	Right F=27.9 (P=<0.0001)	0.24 (0.002)	0.47 (<0.0001)	0.23 (0.0008)		Right F=27.6 (P=<0.0001)	0.06 (0.01)	0.14 (<0.0001)	0.07 (0.0002)

(Threshold p=0.001).

Table 4.3.5: Significant difference in volume in the olfactory regions observed among male age groups.

	Male	Young vs. Middle Mean Diff. (p Value)	Young vs. Elderly Mean Diff. (p Value)	Middle vs. Elderly Mean Diff. (p Value)			Young vs. Middle Mean Diff. (p Value)	Young vs. Elderly Mean Diff. (p Value)	Middle vs. Elderly Mean Diff. (p Value)
Olfactory Cortex	Total F=24.1 (P=<0.0001)	0.21 (0.01)	0.43 (<0.0001)	0.21 (0.0009)	Amygdala	Total F=19.6 (P=<0.0001)	0.06 (0.61)	0.25 (<0.0001)	0.19 (<0.0001)
	Left F=22.1 (P=<0.0001)	0.11 (0.004)	0.21 (<0.0001)	0.09 (0.004)		Left F=18.9 (P=<0.0001)	0.02 (>0.9999)	0.11 (<0.0001)	0.09 (<0.0001)
	Right F=25.03 (P=<0.0001)	0.10 (0.01)	0.22 (<0.0001)	0.12 (0.0003)		Right F=18.6 (P=<0.0001)	0.04 (0.33)	0.14 (<0.0001)	0.09 (0.0001)
Hippocampus	Total F=22.9 (P=<0.0001)	0.21 (0.71)	1.00 (<0.0001)	0.78 (<0.0001)	Orbitofrontal cortex	Total F=38.8 (P=<0.0001)	2.02 (0.007)	5.04 (<0.0001)	3.02 (<0.0001)
	Left F=24.9 (P=<0.0001)	0.09 (0.78)	0.51 (<0.0001)	0.41 (<0.0001)		Left F=39.1 (P=<0.0001)	1.00 (0.01)	2.51 (<0.0001)	1.51 (<0.0001)
	Right F=18.3 (P=<0.0001)	0.11 (0.73)	0.49 (<0.0001)	0.38 (<0.0001)		Right F=37.1 (P=<0.0001)	1.02 (0.01)	2.53 (<0.0001)	1.50 (<0.0001)
Parahippocampus	Total F=24.7 (P=<0.0001)	0.31 (0.40)	1.21 (<0.0001)	0.89 (<0.0001)	Entorhinal Cortex	Total F=35.5 (P=<0.0001)	0.1 (0.29)	0.42 (<0.0001)	0.32 (<0.0001)
	Left F=28.03 (P=<0.0001)	0.15 (0.28)	0.58 (<0.0001)	0.42 (<0.0001)		Left F=41.2 (P=<0.0001)	0.05 (0.19)	0.22 (<0.0001)	0.16 (<0.0001)
	Right F=21.1 (P=<0.0001)	0.15 (0.56)	0.62 (<0.0001)	0.46 (<0.0001)		Right F=28.4 (P=<0.0001)	0.04 (0.46)	0.2 (<0.0001)	0.15 (<0.0001)

(Threshold p<0.001).

Pearson correlation analysis showed a significant correlation between OCV and age, education and TIV for both sexes r=-0.586, r=0.359, r=0.619, females r=-0.629, r=0.311, r=0.470 and males r=-0.641, r=0.401 and r=0.654 respectively (table 4.3.6) (figures 4.3.3 and 4.3.4). In testing for covariance, an ANCOVA test showed that the significant correlation remained in all groups for the OCV when controlling for education

(Women F=27.530, DF=(2,125) p= 0.0001, Men F=17.825, DF=(2,81) p= 0.0001) or for TIV (Women F=66.235 p=0.0001, Men F=49.657 p=0.0001) separately and the significant effect remained in the pairwise tests.

Table 4.3.6: Associations of age, education and TIV with olfactory-related brain regions: Age, education and TIV showed significant correlations with the volume of olfactory regions. Education, however, appeared to have a less strong association that the other two variables

		Age		Education		ICV	
		R^2	P value	R^2	P value	R^2	P value
OCV	**Both sexes**	0.3435	<0.0001	0.1288	<0.0001	0.384	<0.0001
	Women	0.3957	<0.0001	0.09653	0.0003	0.2211	<0.0001
	Men	0.4104	<0.0001	0.161	0.0001	0.428	<0.0001
Hippocampus	**Both sexes**	0.2086	<0.0001	0.07418	<0.0001	0.4974	<0.0001
	Women	0.248	<0.0001	0.04235	0.0193	0.2707	<0.0001
	Men	0.3527	<0.0001	0.1073	0.0022	0.4245	<0.0001
Parahippocampus	**Both sexes**	0.2673	<0.0001	0.09793	<0.0001	0.5085	<0.0001
	Women	0.3651	<0.0001	0.09392	0.0004	0.2904	<0.0001
	Men	0.3758	<0.0001	0.09532	0.004	0.4433	<0.0001
Amygdala	**Both sexes**	0.1693	<0.0001	0.07746	<0.0001	0.6149	<0.0001
	Women	0.2034	<0.0001	0.04546	0.0153	0.4005	<0.0001
	Men	0.3337	<0.0001	0.1175	0.0013	0.5262	<0.0001
OFC	**Both sexes**	0.4159	<0.0001	0.1585	<0.0001	0.3719	<0.0001
	Women	0.4892	<0.0001	0.146	<0.0001	0.1934	<0.0001
	Men	0.5145	<0.0001	0.1631	<0.0001	0.3758	<0.0001
Entorhinal Cortex	**Both sexes**	0.3322	<0.0001	0.1136	<0.0001	0.4028	<0.0001
	Women	0.3819	<0.0001	0.1017	<0.0001	0.2294	<0.0001
	Men	0.455	<0.0001	0.1172	<0.0001	0.3636	<0.0001

95% confidence interval (P<0.05).

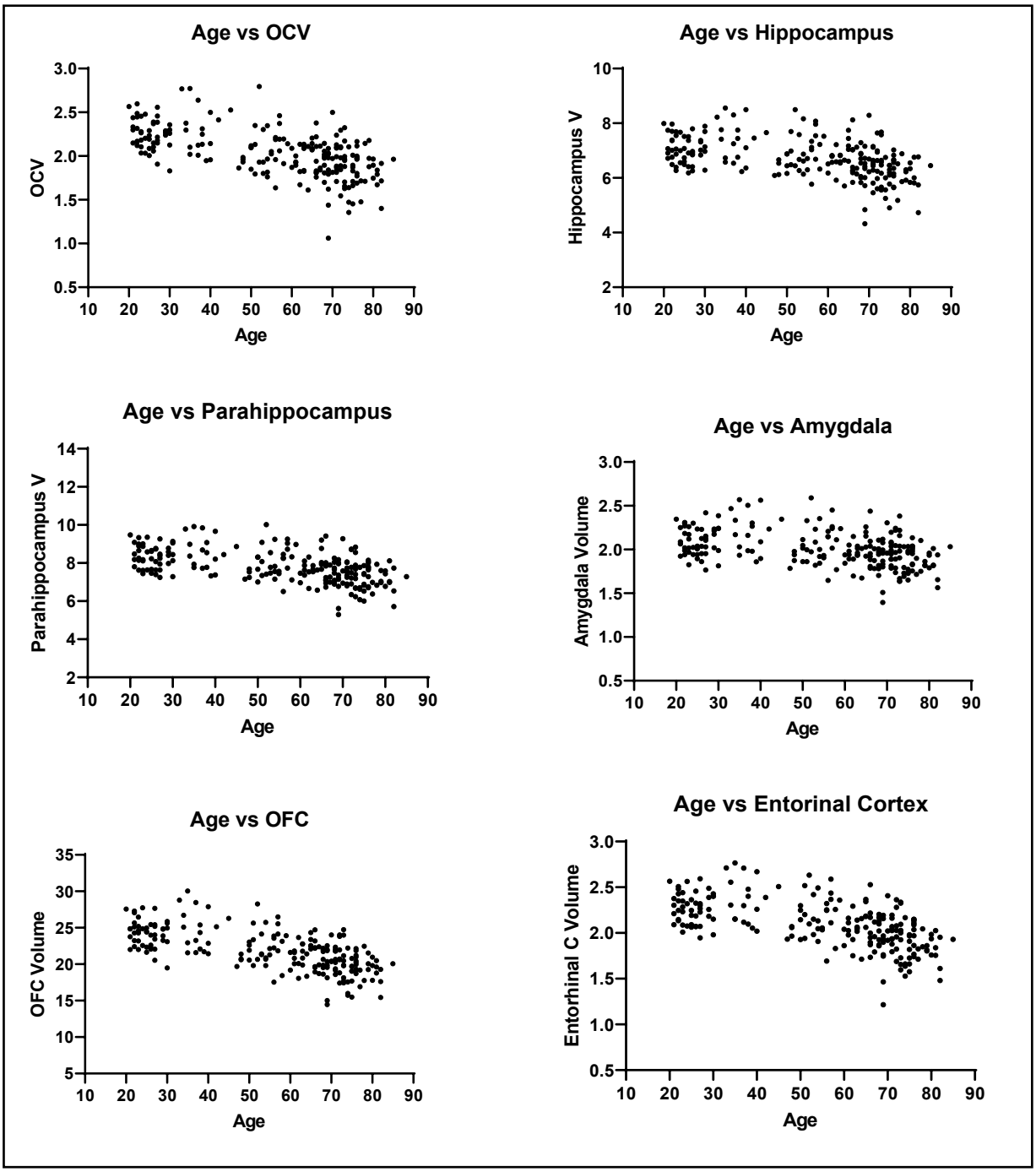


Figure 4.3.3: scatterplot of the association of age with olfactory-related brain regions. Associations found between age (years) with different ROIs. Graphs shows total volume.

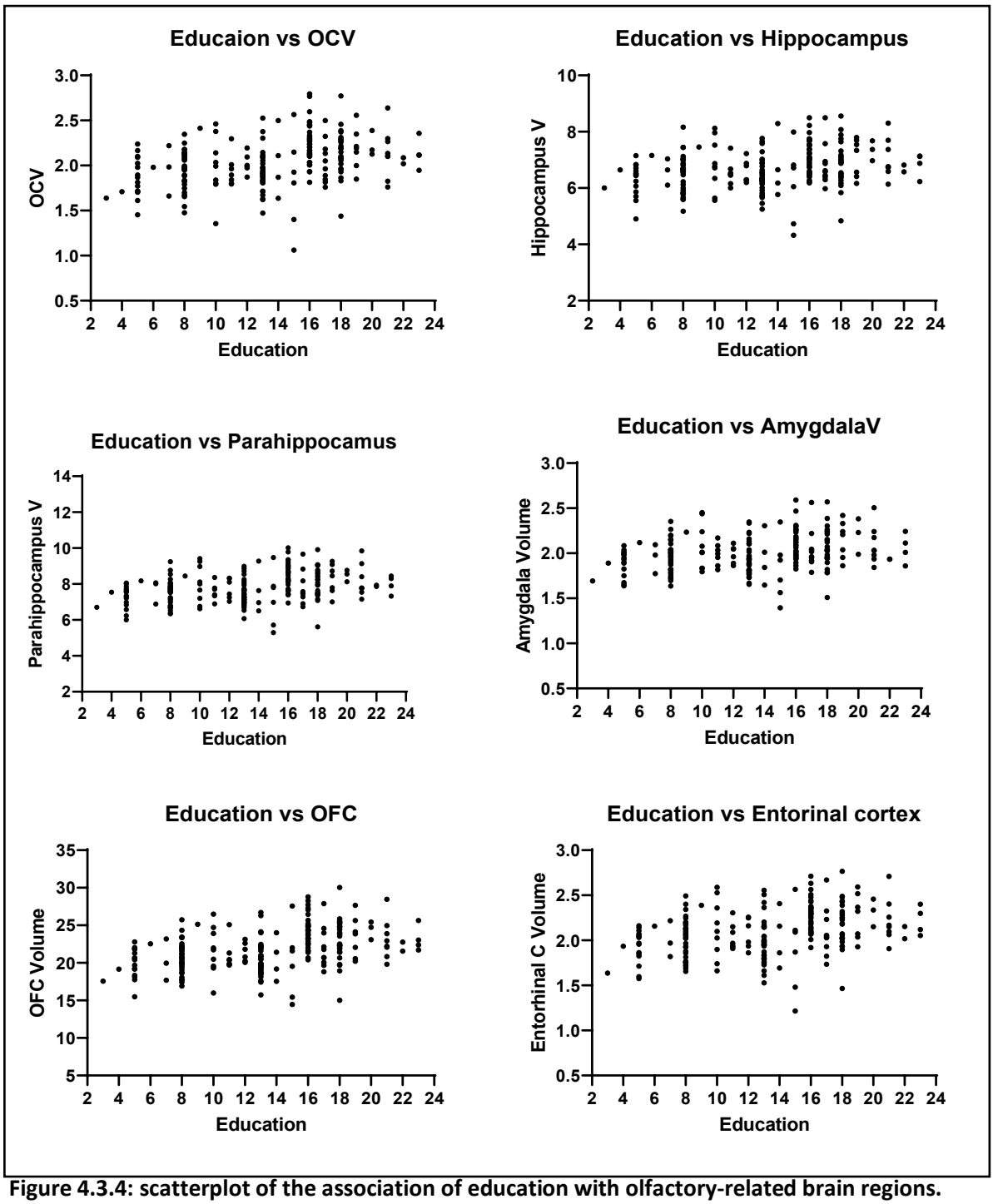


Figure 4.3.4: scatterplot of the association of education with olfactory-related brain regions.
Associations found between education level (years) with different ROIs. Graphs shows total volume.

Hippocampus Volume:

One-way ANOVA analyses showed significant differences in volume between age sub-groups in hippocampus volume (F=30.16, DF =(2,211), p≤0.0001), with the lowest volume observed in the elderly participants. (Figure 4.3.3).

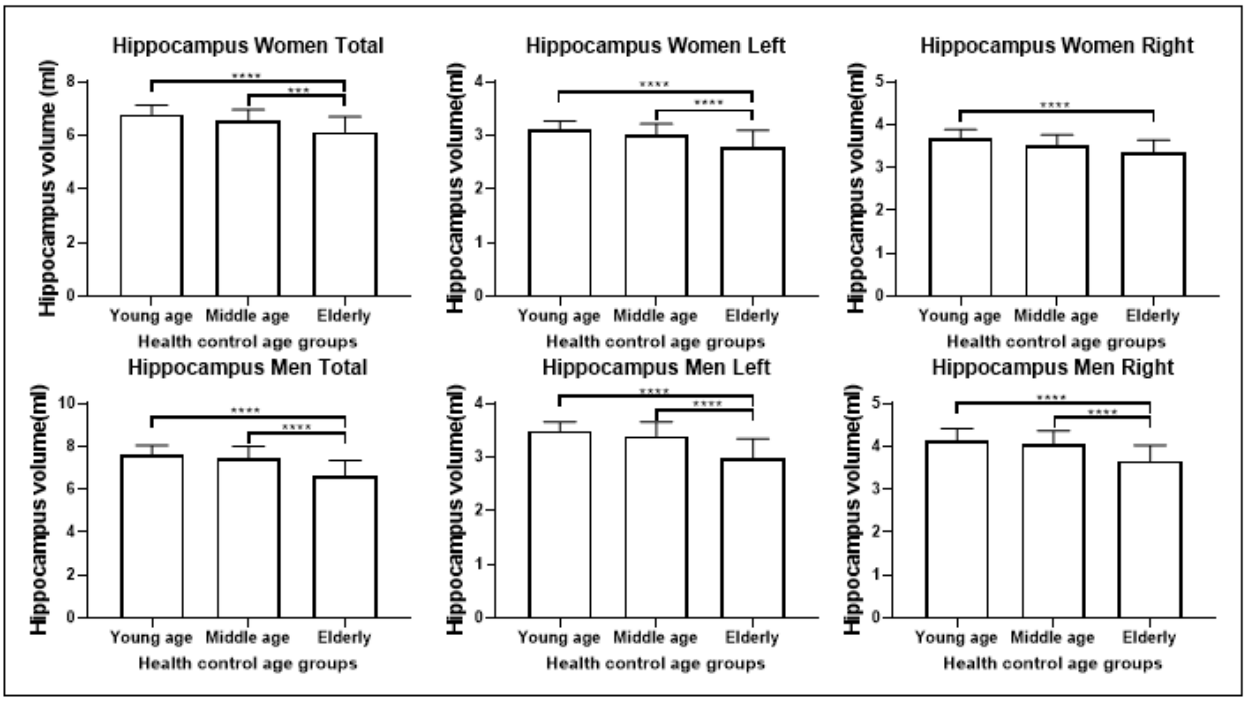


Figure 4.3.5: Hippocampus volume reduction across age groups split by sex: comparative analysis of the hippocampus total, left and right volume respectively in the female population (upper row) and in the male population (lower row).

Post-hoc multiple comparison Bonferroni tests showed significant differences in the volumes of this region in some of the age groups including between the young and the elderly age groups. Hippocampal volume, however, appeared to be sensitive to an age effect in the later part of life, since no significant volume reduction was found between the young and middle age groups (Figure 4.3.3, table 4.3.4 for female and table 4.3.5 for male participants). Correlation analysis showed a significant association between

hippocampus volume and age, education and TIV in both sexes r=-0.456, r=0.272, r=0.705, females r=-0.498, r=0.205, r=0.520 and males r=-0.593, r=0.327 and r=0.651 respectively (table 4.3.6) (figures 4.3.3 and 4.3.4). ANCOVA analysis showed that the significant correlation remained when controlling for education (Women F=19.199, DF=(2.125), p= 0.0001, Men F=18.411, DF=(2,81) p=0.0001) or TIV (Women F=41.916 p=0.0001, Men F=35.423 p=0.0001) separately and the significance remained in pairwise comparisons, except for the volume of the right hippocampus in women where no significant difference was detected between the middle age and elderly groups.

Parahippocampus Volume:

Comparative model analysis showed significant differences in the volume of the parahippocampus among groups (F=38.20, DF =(2,211), p≤0.0001) (figure 4.3.4). The significance among age groups remained when female participants were analysed separately for total volume and left side volume while for the right side volume there was no significant difference found between young and middle aged groups. In male participants, significant differences were also found in all volumes and groups except when comparing the young and middle aged groups (Figure 4.3.4, table 4.3.4 for females and table 4.3.5 for males). A significant correlation was found between parahippocampus volume and age, education and TIV in both sexes: r=-0.517, r=0.312, r=0.713, females r=-0.604, r=0.306, r=0.539 and males r=-0.613, r=0.309 and r=0.665 respectively (table 4.3.6) (figures 4.3.3 and 4.3.4). In the ANCOVA analysis the significant correlation remained when controlling for education (Women F=25.606, DF =(2,126), p= 0.0001,

Men F=20.054, DF =(2,82), p=0.0001) or TIV (Women F=76.408, DF=(2,126), p=0.0001, Men F=42.840, DF=(2,82), p=0.0001), and between group differences remained significant in the pairwise comparisons.

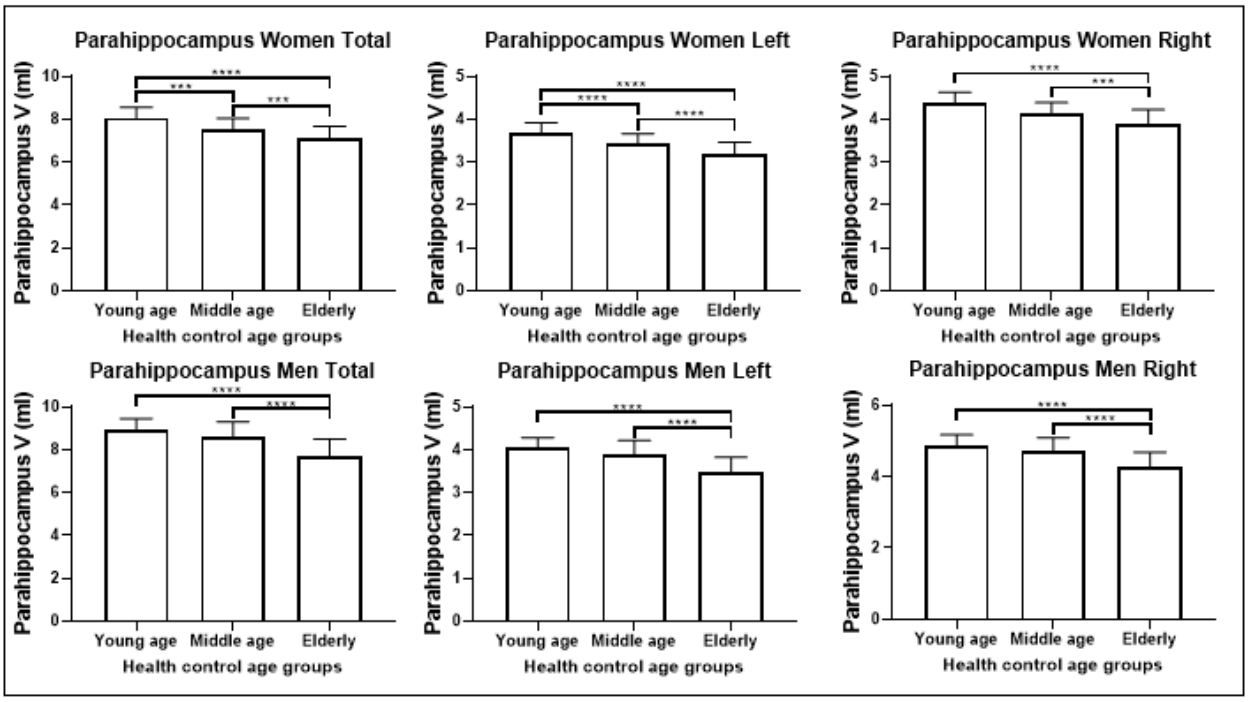


Figure 4.3.6: Parahippocampus volume reduction among groups: parahippocampus volume analysis for total, left and right parahippocampus respectively in the female population (upper row) and in the male population (lower row) showing the amount of significant reduction among the three cognitively healthy groups.

Amygdala Volume:

One-way ANOVA analysis showed significant differences in amygdala volume among age groups (F=21.32, DF=(2,211), p≤0.0001) (figure 4.3.5) with the lowest volume found in the older sub-group. Interestingly, *post-hoc* multiple comparison Bonferroni tests showed significant differences between young and older sub-groups only in the female population. Moreover, significant differences were found between the young and older

sub-groups and between the middle aged and older sub-groups in the male population in the total, left and right volume. The amygdala appears to be more stable in volume in women than in men (Figure 4.3.5, table 4.3.4 for females and table 4.3.5 for males). Significant associations were found between amygdala volume and age, education and TIV for both sexes r=-0.411, r=0.278, r=0.784, females r=-0.451, r=0.213, r=0.632 and males r=-0.577, r=0342. and r=0.725 respectively (table 4.3.6) (figures 4.3.3 and 4.3.4). However, the association with education appeared to be less significant in both female and male participants. Adjusting for education (female F=11.725, DF=(2,125), p=0.0001, male F=15.274, DF=(2,81), p=0.0001) or TIV (female F=38.143, DF=(2,125), p=0.0001, male F=39.475, DF=(2,81), p=0.0001) using ANCOVA analysis showed no influence of these factors as the significance level remained the same even in the pairwise comparisons.

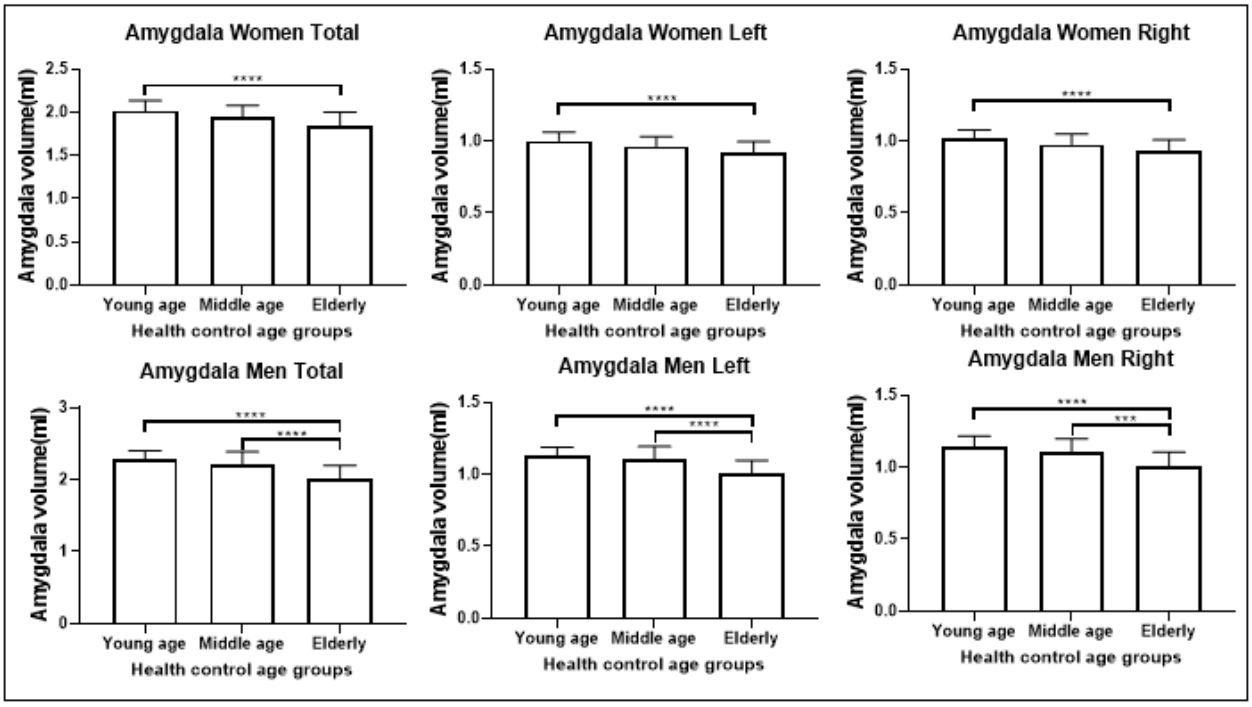


Figure 4.3.7: Amygdala volume differences among age-groups: comparative analysis of the total, left and right hemisphere amygdala volume respectively in the female group (upper row) and in the male group (lower row) in the young, middle and elderly participants; the male population showed a greater ageing effect than females.

Orbitofrontal Cortex Volume:

Group comparison showed significant differences among age groups in orbitofrontal cortex volume (F=68.68, DF=(2,211), p≤0.0001) (figure 4.3.6), with the lowest volumes observed in the older sub- groups. *Post-hoc* analysis showed significant differences between age groups in the female populations for the total, left and right volume. Similar findings were observed in the male population, with the exception of the comparison between the volume of this structure in the younger male sub-group and the middle aged sub-group where no significant reduction was found (Figure 4.3.6, table 4.3.4 for females and table 4.3.5 for males). Correlation analyses showed significant associations between OFC volume and age, education and TIV in both sexes r=-0.645,

r=0.395, r=0.609, females r=-0.699, r=0.382, r=0.439 and males r=-0.717, r=0.403 and r=0.613 respectively (table 4.3.6) (figures 4.3.3 and 4.3.4). Significant differences remained in the ANCOVA and pairwise comparisons when controlling for education (females F=36.168, DF=(2,125), p=0.0001, males F=30.902, DF=(2,81), p=0.0001) or TIV (females F=96.421, DF=(2,125), p=0.0001, males F=77.134, DF=(2,81), p=0.0001) when run separately.

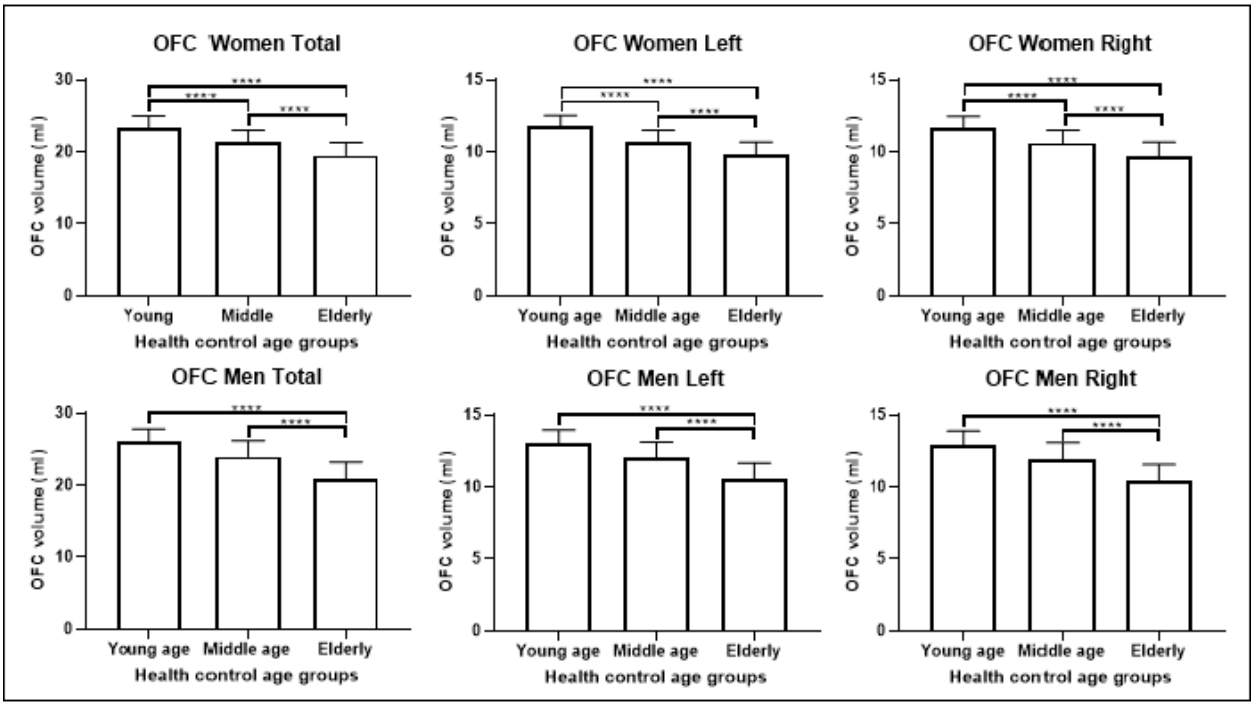


Figure 4.3.8: Orbitofrontal cortex volume differences among age groups in the two sexes: OFC volume comparative analysis among the three age groups for the total, left and right volume in the female population (upper row) and in the male population (lower) showing greater age influence on the OFC volume in the female population as there is significant reduction in volume of this structure also in the middle age group.

Entorhinal Cortex:

One way ANOVA analysis showed significant differences among the three age groups in the entorhinal cortex volume (F=51.66, DF=(2,211), p≤0.0001) with smaller volumes found in the older sub-group (figure 4.3.7). *Post-hoc* analysis showed no significant volume differences between young and middle aged groups both in the female and male participants except for the comparison of the left side volume in female participants. *Post-hoc,* however, when comparing the young sub-group with the older sub-group and the middle aged sub-group with the older sub-group, significant differences were found (Figure 4.3.7, table 4.3.4 for females and table 4.3.5 for males).

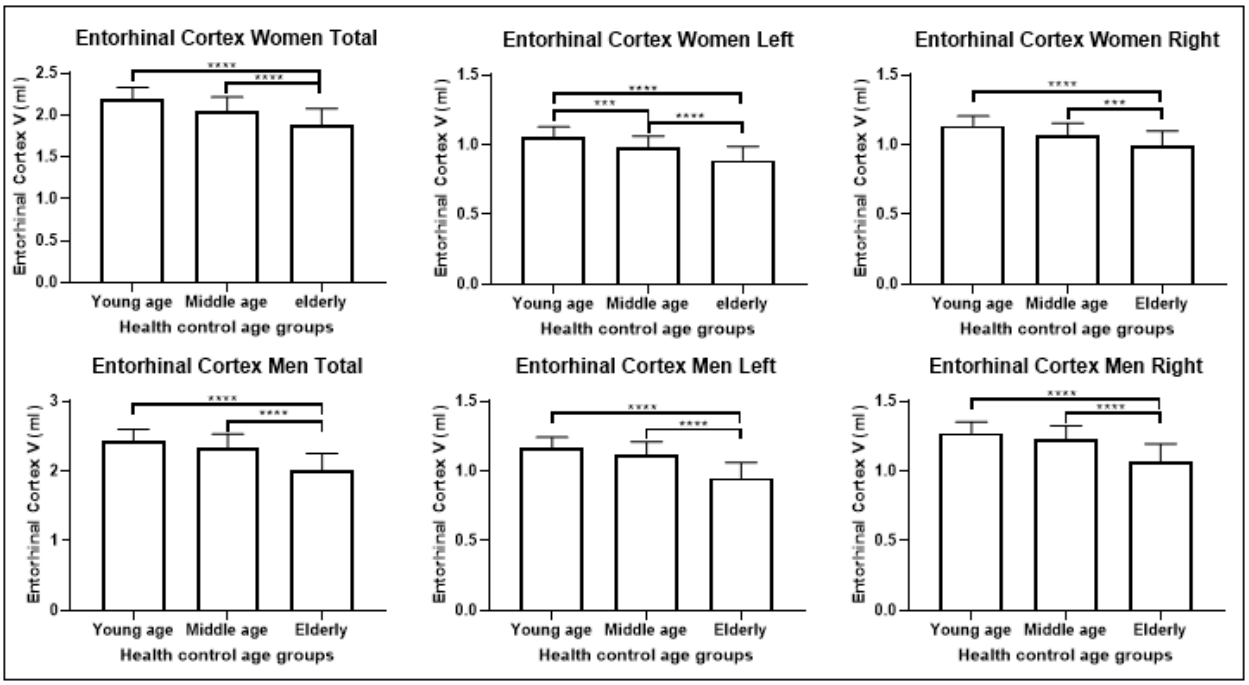


Figure 4.3.9: Entorhinal cortex volume differences among age groups in both sexes: comparative analysis showing entorhinal cortex reduction in the three groups shown for the total, left and right entorhinal cortex volume in the female population (upper row) and in the male population (lower row).

Significant associations were found between the entorhinal cortex volume and age, education and TIV in both sexes r=-0.576, r=0.337, r=0.634, females r=-0.617, r=0.318, r=0.478 and males r=-0.674, r=0.342 and r=0.603 respectively (table 4.3.6) (figures 4.3.3 and 4.3.4). In the ANCOVA with education (females F=25.528, DF=(2,125), p=0.0001, males F=29.290, DF=(2,81), p=0.0001) or TIV (females F=65.567, DF=(2,125), p=0.0001, males F=56.098, DF=(2,81), p=0.0001) as covariates to account for the relative influence of these two variables, the significant differences remained the same and these were also detected in pairwise comparisons.

Testing the effect of the *APOE* ε4 allele:

Comparisons using t-test showed no significant differences between *APOE* ε4 allele carriers and non carriers in total, left and right OC volume (Table 4.3.7). Moreover, using the same test model, no significant difference was observed between *APOE* ε4 allele carriers and *APOE* ε4 allele non-carriers in total hippocampal volume, left and right volume when both sexes were analysed together or separate (Table 4.3.7). This was the case for all volumes, both sexes combined and when volumes were compared in the two sexes separately in total volume, left or right volume for the parahippocampus, amygdala, OFC and entorhinal cortex (Table 4.3.7).

Table 4.3.7: Comparative analysis of olfactory-related brain regions between *APOE* ε4 carriers and non-carriers showed no significant differences in any regions in both sexes.

	OCV		Hippocampus		Parahippocampus		Amygdala		OFC		Entorhinal	
	t	p	t	p	t	p	t	p	t	p	t	p
Total volume	0.91	0.37	0.78	0.44	0.59	0.56	0.79	0.44	0.71	0.48	0.98	0.33
Left volume	0.99	0.33	0.48	0.63	0.48	0.64	0.42	0.68	0.49	0.62	0.79	0.43
Right volume	0.82	0.42	0.94	0.35	0.65	0.52	1.1	0.28	0.89	0.38	1.1	0.28
Women total	2.02	0.06	1.65	0.12	1.55	0.14	1.87	0.08	2.13	0.052	2.02	0.064
Men total	0.601	0.56	0.203	0.84	0.19	0.85	0.52	0.61	0.31	0.76	0.016	0.994

Significant level = $p<0.001$

4.3.4 Discussion

This cohort study investigated alterations of volume in olfactory-related brain regions and their associations with age, education and TIV in neurologically healthy adults. The use of MRI imaging techniques enabled the study objectives to be met. Our study showed that loss of volume in olfactory-related brain regions in men occurs approximately around the seventh decade of life while in women is detectable as early as the fourth decade except for the volume of the hippocampus and amygdala that became noticeable at around the seventh decade in women. This finding confirms what had been suggested by an earlier study that reported that women have greater brain atrophy than men (Jiang et al., 2014). In detail, variability in volumetric decline of olfactory-related brain regions was seen in this study. Women appeared to have earlier volumetric loss in the olfactory cortex as significant differences from the youngest age group were detectable in both hemispheres in the comparison with the middle aged sub-group. In men, the volume of the OC on the right side showed a significant decline between the middle aged and older sub-groups, while on the left, reduction in volume was only seen after the age of 65 years. This observation does not find any support in earlier studies that reported no volumetric reduction associated with age (Kondo et al., 2020). For the hippocampus, women showed less volumetric reduction on the right side since no significant differences were observed between the middle aged and the older sub-groups while on the left side a significant volumetric reduction was observed after 65 years. In men, a significant volumetric reduction was observed in the hippocampus in the older age sub-group. The

greater volumetric reduction on the left side had already been reported previously by a study that found a significant association between biological factors and volume of the left hippocampus and amygdala in both sexes (Orihashi et al., 2020). This finding supports earlier evidence that the right hippocampus shows less volume loss in general (Frisoni et al., 2008) and findings of a large study that found that reduction in hippocampal volume starts in the seventh decade of life in both sexes (Nobis et al., 2019). In general, hippocampal volume alterations are associated with ageing (Scahill et al., 2003). As for the parahippocampus, volumetric loss on the left side is seen earlier in female participants in line with the evidence reported in the literature previously (Jiang et al., 2014). Significant reduction in volume was noted when the young age sub-group was compared with the middle aged sub-group. However, in the male participants volumetric reduction was similar to that observed in the hippocampus where substantial volumetric reduction is seen after the age of 65 years. The amygdala, however, seems to undergo a subtler and more gradual decline than other structures in female participants, with volumetric reduction becoming significant only when the two more extreme age sub-groups (young and older) were compared. Instead, in the male participants volumetric reduction was seen at a later point with differences becoming significant only in the comparison between middle aged and older age sub-groups and being more substantial on the left. This finding is in line with those of a recent report that volume of the left amygdala is reduced in older people (Iizuka et al., 2021). For the OFC, volumetric reduction was greater in women and detectable already in the middle aged sub-group comparison with the young sub-group, while in men the volume of this brain region appeared more stable up to an older age,

with volumetric reductions becoming significantly detectable only when the middle aged sub-group was compared with the older sub-group. This finding is in line with earlier observations that led the authors of the study to suggest that volume loss in this region becomes noticeable after the age of 40 years (Shen et al., 2013). In the entorhinal cortex, volumetric loss on the left was noticeable very early in women and differences appeared significant in group comparisons with the young age sub-group. In men, however, volume loss appeared to accelerate later on in life with significant differences seen between the middle aged and older age sub-group comparisons. The pattern of reduction observed for this structure is similar to that seen in the parahippocampus as there is a close connection and direct influence of the parahippocampus on entorhinal cortex (Iizuka et al., 2021).

The volumetric loss seen in olfactory-related brain regions may be part of the overall grey matter loss experienced by people as progressive grey matter shrinkage has been reported after the second decade of life (Shen et al., 2013). Regional and global brain volume loss is strongly associated with the brain ageing process (Peters, 2006; Scahill et al., 2003). In parallel, olfactory dysfunction is associated with volume reduction in olfactory cortex, hippocampus, parahippocampus and orbitofrontal cortex (Bitter et al., 2010). The finding of the present study, therefore, could explain the olfactory deficits observed in the ageing population. Moreover, the present study results confirmed earlier evidence that parahippocampus and entorhinal cortex show greater volume loss with age but that is not the case for the hippocampus and amygdala (Sele et al., 2021).

Different factors might be involved in the brain regional alterations observed in this study and these could also include the potential consequences of stress (Lindgren et al., 2016). In addition, neurotransmitters' level, such as dopamine and serotonin, decline with ageing and might cause brain regional alterations (Peters, 2006). For example, dopamine level decreases approximately 10% per decade from early adulthood (Mukherjee et al., 2002). An fMRI study demonstrated a significant decrease in brain activities in regions of the olfactory cortex, hippocampus, parahippocampus, orbitofrontal cortex and entorhinal cortex in older adults compared with young adults (Wang et al., 2005). The present study indicates that women show greater brain volume reduction than men. This finding is in line with a similar observation that has been previously reported (Crivello et al., 2014; Guo et al., 2016).

In the sub-group for whom genetic status was available, no significant effect of the *APOE* ε4 genotype was found on the volume of olfactory-related brain regions. This negative finding could be explained by several factors. First, it could be related to the fact that none of the *APOE* ε4 carrier participants in the present study was homozygous, while an earlier study had found a significant effect of *APOE* ε4 on brain regional volume and reported greater hippocampus volume loss in homozygous *APOE* ε4 carriers than in heterozygous or non carrier participants (Crivello et al., 2010). It is also important to mention that in volume comparative analyses, a 99.9% confidence interval threshold was applied as recommended for neuroimaging studies (Woo et al., 2014), even if a VBM

analysis method was not used in this study. In the present study, brain regional volumes were not normalised to TIV, but TIV was used as a covariate in the analyses. Previously published studies have reported no significant difference between normalised and non-normalised brain regional volumes, especially for the hippocampus (Embong et al., 2013; Jalaluddin et al., 2013). Overall, grey matter showed significant alterations in all *post hoc* analyses in women and men and this was the normal outcome in relations to the alterations found in olfactory-related brain regions. However, no alterations were found in white matter except for the volumes of the older male sub-group only.

In this study we investigated regional volumes not the pathways. Thus, the ROIs included in this study were identified using the atlases mentioned in the method section. To the best of our knowledge this is the first study of its kind as none of the earlier studies has carried out a large and comprehensive study of most parts of olfactory-related human brain regions for all age groups, taking sex into account and also by taking volumetric measurements of both brain hemispheres using MRI imaging technology.

Because of the wide variability of brain alterations in people undergoing normal ageing, finding a consistent pattern of atrophy may be difficult and might need more sophisticated investigations. More research is needed to comprehend fully the trajectories of atrophy in distinct brain regions involved in olfactory processing, as well as investigating volumetric loss both in normal ageing populations and in disease situations. Taken together, however, the results of this study suggest that monitoring of volume of olfaction-related brain regions in the ageing population may be valuable to detect risk of neurodegenerative diseases.

4.4 Chapter discussion and conclusion

The results of this chapter indicate that a large proportion of the mentally healthy elderly population has a weak sense of smell. Not only that, but most of them are not aware of having a weak sense of smell, and this certainly poses a threat to their lives and the lives of others around them. It is important to stress that the self-report olfactory questionnaire is not sufficient to test olfactory function among the elderly as no association was found with scores obtained on the objective olfactory test. This means that health care providers are required to use objective olfactory tests when testing elderly individuals and not only ask them if they are experiencing any problems with their sense of smell. The studies reported above also found that there is a relationship between sense of smell and cognitive abilities in older women, indicating that detecting impairment in someone's sense of smell might predict that they might be at risk of future development of a neurodegenerative condition if a weak sense of smell is observed in association with a degree of cognitive decline. It is important to differentiate between an isolated olfactory dysfunction that may be caused by ageing, and an indication of a potential neurodegenerative disease, when an olfactory deficit is associated with cognitive impairment and the detection of such an association should prompt additional tests in an elderly individual.

The third experiment in this chapter showed that there is a reduction in the size of all those regions of the brain related to olfactory processing as part of the process of normal ageing. Again, this regional volumetric reduction seems to be more accentuated

in women as it is already detectable in middle aged individuals while in men it becomes noticeable only at a more advanced age.

This fourth chapter, which includes clinical experiments on cognitively healthy populations, clearly shows that age has an effect on the sense of smell. This is based on objective tests of sense of smell or through magnetic resonance imaging of the regions of the brain related to the processing of olfaction. The findings of this chapter show clearly that the effect is greater in women, as a relationship was found between a weak sense of smell and a lower cognitive performance in those women who were smokers. Moreover, the reduction in size of those brain regions associated with olfactory processing was also greater in women. As mentioned earlier, women are more at risk of developing neurodegenerative diseases and the findings in this chapter lend additional support to them being at greater risk.

There are several factors that may lead to a loss of the sense of smell in the elderly, including infection, head trauma or else. It is important to pay attention to an elderly individual's olfactory capacity in clinical setting and to include objective testing of this function since the findings of the studies presented in this chapter suggest that this assessment in combination with cognitive assessment might alert to future neurodegeneration. Detection of olfactory dysfunction at an early stage could potential help reducing the extent of these deficits and implement potential treatments.

4.5 Limitations:

As for the overall potential limitations of the studies presented in this chapter, the biggest disadvantage is that in this chapter two study samples were assessed, one in whom we tested olfaction abilities and the other in whom we obtained regional volumetric values for olfactory related brain regions using neuroimaging. However, the results of this chapter are complementary and show a greater effect of ageing in women than in men either in olfactory performance when correlated with cognition levels or in volumetric measurements of olfaction-related brain regions.

To investigate the predictive value of olfactory dysfunction for cognitive decline, one limitation of the studies in this thesis is that there was no evaluation of hearing status as the cognitive test final scores could have been affected by hearing loss as previously reported (Pachana et al., 2006). The telephone-based cognitive test used in the first two experiments examined mainly amnestic cognition but not non-amnestic cognition. In other words, the approach used can provide a memory composite score where there are other cognitive domains that could also be affected (Duff et al., 2015). This study's cognitive evaluation was restricted to a screening battery t-MMSE and TICS. A more extensive neuropsychological examination would have been more appropriate for getting a better appraisal of cognitive status. However, a part from being less expensive, the t-MMSE and TICS have the advantage that these tests could be administered either by telephone or face to face and can be valid alternatives to the standard MMSE (Duff et al., 2015; Fong et al., 2009; Newkirk et al., 2004). A comprehensive neuropsychological battery was used in the sample studied in experiment 3, assessing a range of cognitive domains in depth.

However, given the retrospective nature of this analysis, no olfactory tests were available for this sample.

Furthermore, individuals differ in brain size and that may affect the outcome of the study. However, this potential issue was minimised by using TIV values as a covariate in the analyses.

A further limitation is the cross-sectional design used in this study. Indeed, longitudinal studies are the best way to assess individual brain alterations. Also, in comparing olfactory-related regional brain volumes, no gap between age sub-groups was introduced to ensure that any potential change in volume would be noticeable. The findings of the study appear to indicate that volume reduction can occur gradually across the lifespan in some regions and more substantially in the later part of life for other regions.

Different olfactory region terminology and anatomy have been introduced in the literature previously. For example, Wilson (2009) stated that the olfactory cortex includes the piriform cortex (also called pyriform or prepyriform cortex) that is the largest sub-region of the olfactory cortex (Wilson, 2009). In another example, Doty (2017) defined the primary olfactory cortex as including the anterior olfactory nucleus, pyriform cortex, periamygdala and amygdala complex regions and rostral entorhinal cortex (Doty, 2017). Moreover, another study considered primary olfactory cortex as including the piriform cortex associated with the anterior olfactory nucleus, the anterior perforated substance, olfactory tubercle, anterior portion of periamygdaloid cortex and amygdala (Wang et al., 2010). These different terminologies and anatomical regions of the olfactory cortex or

primary olfactory cortex could be misleading and include noise in the data and disrupt the processing flow of research involving olfactory function. However, this potential limitation does not influence our study since we investigate volumetric alterations of olfactory related brain regions not the process or function of those regions, nor perception of an odorant.

Chapter 5: Atrophy of olfactory-related brain regions in MCI and AD dementia

5.1 Experiment 4: Atrophy of the Olfactory Cortex in Alzheimer Disease

5.1.1 Introduction

Numerous health problems occur with ageing such as cognitive decline, cardiac disorders and/or neurodegenerative diseases including Alzheimer's disease (AD) (Cermakova et al., 2015). Neurodegenerative diseases, including AD, Parkinson's disease (PD) and Huntington's disease (HD) among others, are progressive disorders that lead to neuronal loss and consequent structural alterations in the brain (Gao & Hong, 2008). AD is a chronic neurodegenerative disorder that affects millions of individuals worldwide and is one of the most debilitating type of dementia that contributes up to 60 – 70% of the cases worldwide based on data by the World Health Organization (de la Monte & Wands, 2004; *Dementia*, 2017; Niikura et al., 2006). Symptoms include memory dysfunction and deficits in attention, planning, language, and overall cognitive function (Albert et al., 2011).

Some studies suggest that olfactory dysfunction is also a common symptom of neurodegenerative disorders including AD (Doty et al., 1991; Ruan et al., 2012) and evidence supports that it can be an early marker of this disease (Doty, 2009). Indeed, several studies have shown that when compared with age-matched controls it is evident that olfaction is affected in the early stage of AD (Djordjevic et al., 2008; Godoy et al.,

2014). Olfactory dysfunction is also observed in individuals with Mild Cognitive Impairment (MCI), some of whom will progress on to AD dementia (Morris et al., 2001). The sense of smell also declines in otherwise neurologically healthy elderly people (C. Zhang & Wang, 2017). However, this is said to be accelerated in individuals with a neurological disease (Godoy et al., 2014). Significant associations have also been found between performance on odour identification tasks and cognitive scores (Larsson et al., 2000). These data suggest that levels of olfactory function may be a marker of impending AD and should be assessed, along with other markers, in ageing individuals.

Olfactory dysfunction may have a number of causes, such as nose or brain injury, age-related structural alterations in the olfactory system, environmental, or other biological alterations that reduce the efficiency of smelling (Doty, 2017). Given the olfactory dysfunction observed in AD, it would be reasonable to expect that olfactory structures in the brain may be affected (Pearson et al, 1985). Indeed, some studies have found that AD individuals have atrophy of the olfactory epithelium (Serot et al., 2003), olfactory bulb and tract (Thomann, et al., 2009), and of the primary olfactory cortex (POC) (Vasavada et al., 2015). Atrophy in AD is also observed in other brain regions including the hippocampus (Pearson et al., 1985), basal forebrain, thalamus and amygdala (Pini et al., 2016). However, in the medial temporal lobe, AD pathology and volume loss appear earliest in the entorhinal cortex, an area within the POC (Devanand et al., 2012; Du et al., 2004). Thus, while many studies indicate that hippocampal atrophy is the best core marker for AD, measurements of the entorhinal cortex may provide the earliest detection

(Dubois et al., 2007; Johnson et al., 2012; Pini et al., 2016; Sperling et al., 2011; Henneman et al., 2009).

Despite the fact that accumulation of β-Amyloid occurs in AD brains, and accumulation of this protein in the olfactory bulb is one of the earliest sites of pathology in AD (Christen-Zaech et al., 2003; Thomann, et al., 2009), it has been shown that β-Amyloid burden is not directly related to olfactory deficits (Bahar-Fuchs et al., 2010; Wilson, Arnold, et al., 2007). Furthermore, β-Amyloid accumulation is frequently observed in individuals without AD (Carvalho et al., 2018). In contrast to β-Amyloid accumulation, neurofibrillary tangles do appear to affect olfactory function (Ruan et al., 2012; Wilson, Arnold, et al., 2007). These tangles are detected in the olfactory bulb and primary olfactory sensory cortices in human AD brains (Arnold et al., 2010). Moreover, neurofibrillary tangles in AD are found in the area of olfactory processing (Bahar-Fuchs et al., 2010; Kovács et al., 2001). Other molecular depositions in the AD olfactory bulb are also observed including accumulation of tau proteins that correlates with AD stage (Lazarov & Marr, 2010) and Caspase activation (Foveau et al., 2016). Caspase-6 activation, which occurs early in the pathogenesis of AD (Flores et al., 2018; LeBlanc, 2013; Ramcharitar et al., 2013), is observed in the olfactory bulb of patients with AD and its activity is associated with Tau pathology but not β-Amyloid accumulation (Foveau et al., 2016).

Structural and functional brain asymmetry has been observed in humans and animals (Hugdahl, 2005; Sabine et al., 2011; Toga & Thompson, 2003). Of note, POC volume decreases have been observed among AD patients on both sides of the brain (Vasavada et al., 2015). However, using metabolic measures such as 18-F-

fluorodeoxyglucose-PET or MRI, it has been shown that in AD brain regional alterations in the left hemisphere are more severe and occur prior to atrophy in the right hemisphere (Donix et al., 2013; Thompson et al., 2007; Toga & Thompson, 2003). Aside from the lateral asymmetry, grey matter concentration in the olfactory system also differs according to sex. For example, women have higher grey matter concentration in orbitofrontal cortex and right amygdala and hippocampus, while men have higher grey matter concentration in left entorhinal cortex and left dorsal insular cortex (Garcia-Falgueras et al., 2006), an observation that supports morpho-functional differences in olfactory neuronal networks between men and women.

A number of studies has shown atrophy of the entorhinal cortex in AD (Devanand et al., 2012). However, there is a lack of literature more specifically on OC volume in AD, and in particular if there are sex-specific effects. In this study, the aim was to determine if there were atrophy of the OC in a cohort of individuals with AD, and if so, how sex, age and level of education may influence variance in volume in structures constituting the primary OC. Furthermore, a secondary aim was to determine if there were any a/symmetry of these brain regions.

5.1.2 Method and materials

Participants:

The samples included in the present case-control study included 25 cognitively healthy older adults (14 women and 11 men) and 14 individuals with a diagnosis of AD

(9 women and 5 men) from an observational study previously described in Croteau et al. (2017) (Croteau et al., 2017). All participants provided written informed consent prior to study entry. Ethical approval for this study was obtained from the ethics committees of the Research Centre on Aging - CIUSSS de l'Estrie – CHUS (2009-111 and 2010-163). Probable or possible AD was defined using the National Institute on Aging - Alzheimer's Association (NIA-AA) criteria (McKhann et al., 2011) with or without the use of prescribed medication for the AD patients (Castellano et al., 2017).

MRI screening:

Participants were assessed using a 1.5 Tesla magnetic resonance imaging scanner (MRI Sonata, Siemens Medical Solutions, Erlangen, Germany). A T1-weighted sequence (TR=16.00ms, TE=4.68ms, field of view=256 x 240 x 192 mm, matrix size= 256 x 256 x 164, flip angle=20° and 1mm isotropic voxels) was acquired. Volumetric MR imaging measurements were performed using the PNEURO tool implemented in PMOD 3.8 (PMOD Technologies Ltd., Zurich, Switzerland). Brain anatomy was automatically segmented into 119 volumes of interest according to the Automatic Anatomical Labelling atlas [AAL atlas; (Tzourio-Mazoyer et al., 2002)]. We considered the OC regions in the brain as defined by (Dejerine, 1895) and as used in a recent study (Fjaeldstad et al., 2017). The OC includes the olfactory tract, amygdala, piriform cortex, anterior perforated substance, the subcallosal area (including the subcallosal cingulate gyrus) and the anterior cingulate cortex.

Statistical analysis:

OC and hippocampal volumes obtained from three dimensional structural MRI scans, and the assessment of a/symmetry, were compared using an unpaired t-test. Pearson's correlation tests were used to determine if there were a correlation between age and OC or hippocampal volume in the control and AD participants. Pearson's correlation tests were also used to determine if there were a correlation between education levels and these brain regions. The unpaired t-test and correlation analysis were performed using Graphpad Prism 7 (GraphPad Software San Diego, CA, USA). SPSS software (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY) was used for the linear multiple regressions. Raw OC and hippocampus volume data were the dependent variables and AD, sex, age and TIV were used as independent variables. The level of significance was set at $p < 0.05$.

5.1.3 Results

Demographics:

Table 5.1.1 shows the demographic information including cognitive screening scores and education levels for the control participants (14 women, 11 men) and AD individuals (9 women, 5 men).

Table 5.1.1. Demographic Profiles.

		Control (n=25, M=11, F=14)				AD (n=14, M=5, F=9)				p value
		Min	Max	Mean	SD	Min	Max	Mean	SD	
Men + women	Age (y)	65	86	71.1	5.22	65	82	75.6	4.60	0.047*
	MMSE score	27	30	29.3	0.89	19	29	25.4	2.96	<0.0001*
	Education (y)	7	22	14.8	4.54	8	22	12.6	4.72	0.181
Men	Age (y)	65	85	71.0	6.68	70	76	72.6	2.41	0.613
	MMSE score	28	30	29.5	0.69	23	29	25.3	2.87	0.0004*
	Education (y)	13	22	17.1	2.88	10	22	17.3	5.12	0.940
Women	Age (y)	65	78	71.2	3.99	65	82	75.6	5.27	0.03*
	MMSE score	27	30	29.1	1.03	19	29	25.4	3.17	0.0005*
	Education (y)	7	19	12.9	4.84	8	16	10.6	2.83	0.200

MMSE: Mini-Mental State Examination

There was a subtle yet significant difference in age between control participants and AD individuals (t=2.05, p=0.047). This was mainly driven by a difference in age between the female controls and AD participants (p=0.03). There was no difference in age between the male controls and AD participants. As expected, the Mini-Mental State Examination (MMSE) scores showed significant differences between the two groups (p<0.0001). For education levels, there was no significant difference observed between the control and AD participants. However, the male participants (control and AD combined) had higher education levels overall than the female participants (control and AD combined) (p=0.0004).

Atrophy of the olfactory cortex in AD

The MRI findings demonstrated that total OC volume was significantly smaller in AD when compared with control participants (men and women combined, p=0.009, Fig.5.1.1A). This difference in volume was most obvious in the left OC volume (p=0.003, Fig.5.1.1B) with only a trend difference in the right OC volume (p=0.06, Fig.5.1.1C).

In order to determine if both sexes showed OC volume atrophy, we assessed this separately for men and women. Despite the small sample size, a significantly smaller total OC volume was found in men with AD when compared with control participants (9.3%, p=0.045). This difference was more pronounced in the left OC volume compared with the right (left, 11.45%, p=0.02; right, 7.34%, p=0.12). In women AD participants there was a trend difference in total OC volume (6.8%, p=0.06), and a significant difference in the left OC volume (10%, p=0.04). The right OC volume showed no difference in women between control and AD participants.

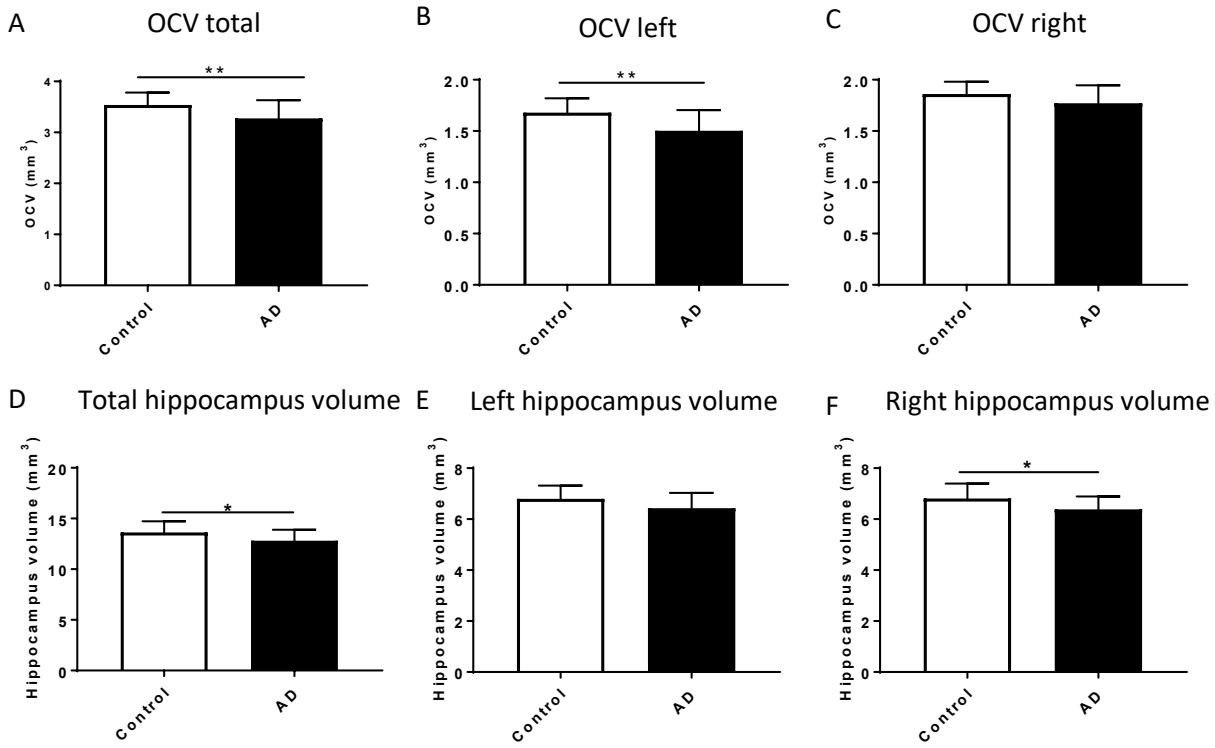


Figure 5.1.1: Olfactory cortex and hippocampal atrophy in AD. A) Comparison of the total OC volume between AD and control when males and females are combined; B) Comparison of the left OC volume between AD and control when males and females are combined; C) right olfactory cortex volume in the AD and control group (males and females combined); D) Comparison between AD and control in total hippocampus volume in males and females combined; E) left hippocampus volume in AD and control groups in males and females combined; and F) right hippocampus volume in AD and control groups in males and females combined.

As expected, there was a significant difference in the total volume of the hippocampus in the AD participants when compared with the control group (men and women combined, p=0.03, Fig.5.1.1D). This was more pronounced in the right hippocampal volume (p=0.03, Fig.5.1.1F) than the left hippocampal volume (p=0.055, Fig.5.1.1E). When assessing volume of this structure by sex, male AD participants showed a significant difference in the total hippocampal volume (9.2%, p=0.026) and in

the right hippocampus volume (10.7%, p=0.011). No significant difference in hippocampal volume was observed in female AD participants when compared with controls.

Region specific brain asymmetry

Structural, functional and behavioural brain asymmetries have been observed in humans for a number of brain regions (Toga & Thompson, 2003). However, there is limited data on the OC. A significant asymmetry was observed in our cohort in the OC of controls when comparing the left and the right volumes ($p<0.0001$, Fig.5.1.2A). A similar asymmetry was observed in the OC of the AD participants ($p=0.0009$, Fig.5.1.2B). In both groups, the left OC volume was significantly smaller than the right side (9.8% in the control group and 15.1% in AD patients). This asymmetry was observed in both the male ($p=0.003$) and female ($p=0.0009$) control participants, and in the female AD participants ($p=0.005$). However, it was not detected in men with AD. In all cases where asymmetry was observed, the left OC volume was smaller than the right one.

In sharp contrast to the asymmetry observed in the OC volume, no asymmetry was observed in our cohort in the hippocampus when comparing left versus right volumes in the control group (Fig.5.1.2C) or in the AD group (Fig.5.1.2D). A similar result was found when the groups were separated based on sex.

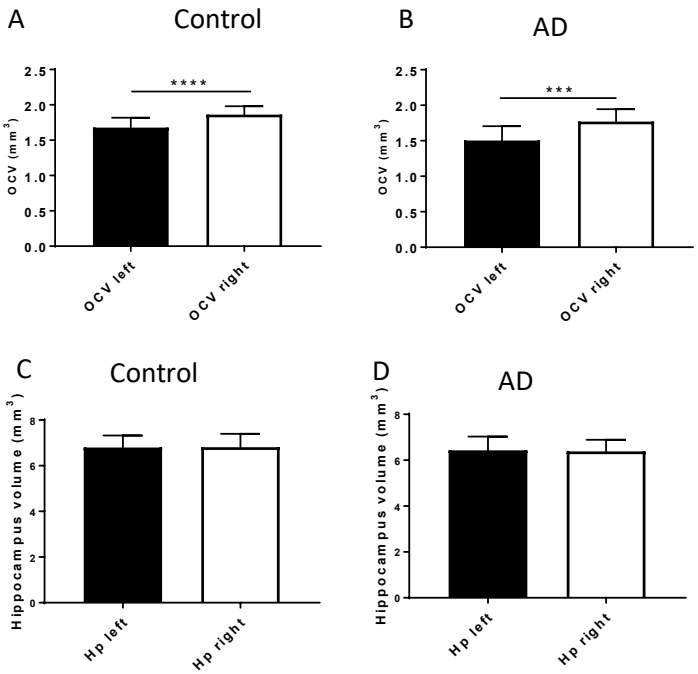


Figure 5.1.2: Select regions of the brain that show hemispheric asymmetry. A) OCV asymmetry in the control group when males and females are combined; B) OCV asymmetry in the AD group when males and females are combined; C) No hippocampus volume asymmetry in the control group when males and females are combined; D) No hippocampus volume asymmetry in the AD group when males and females are combined.

Age associated decrease in olfactory cortex volume in AD

We first assessed if there were a correlation between left or right OC volume and age in controls and AD. No correlation between the left olfactory cortex volume ($r=0.2099$, $r^2=0.0441$, $p=0.314$) or the right ($r=0.1803$, $r^2=0.032$, $p=0.389$) and age was found in controls when males and females were combined ($DF=24$). Comparing the slopes of the left and right OC volume in the control participants in relation to age (men and women combined) revealed no significant difference between the slopes ($p=0.836$) (Fig.5.1.3A). A similar finding was observed in the AD participants where no correlation was found between left ($r=0.4526$, $r^2=0.205$, $p=0.104$) or right OCV ($r=0.1803$, $r^2=0.006$, $p=0.795$) and age as well as when we compared the slopes ($p=0.292$, $DF=13$) (men and women combined, Fig.5.1.3B). We then assessed age in relation to the left OC volume between the control and AD groups. There was a significant difference between the two slopes in the left volume between AD and control with age ($p=0.03$, Fig.5.1.3C). The OC age related volume loss was greater in the AD participants than in the control participants. This was not observed when comparing age and the volume of the right OC in control and AD participants (Fig. 5.1.3D).

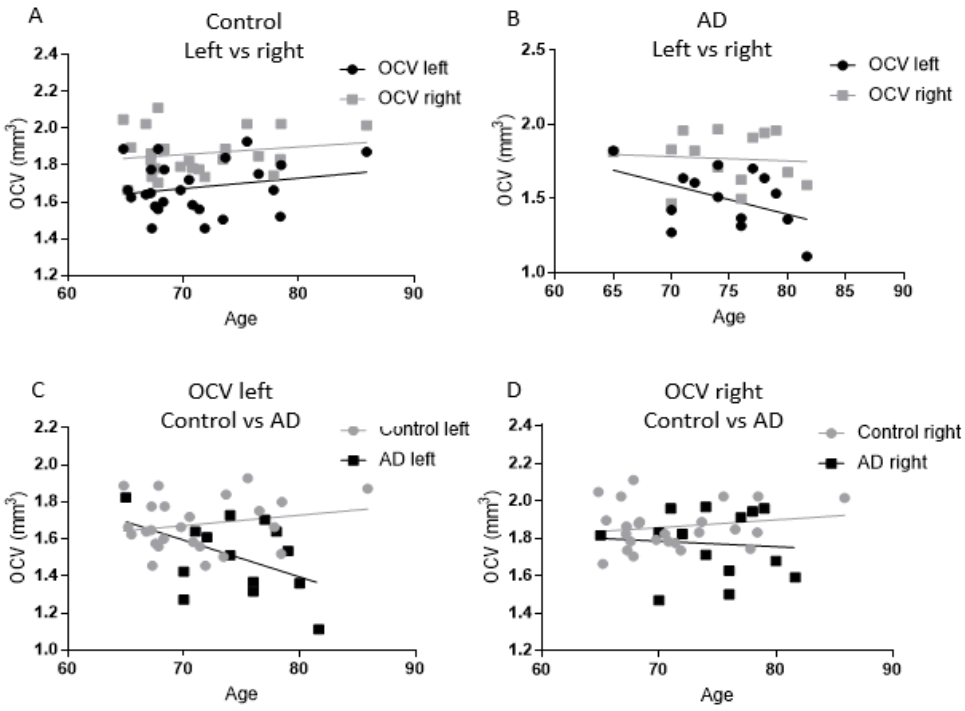


Figure 5.1.3: The relationship between olfactory cortical volume and age in the AD and control participants. A) No correlation in controls between the left (black) or the right (grey) olfactory cortex volume and age (x-axis) when males and females were combined. B) No correlation between the left or right olfactory cortex volume and age (x-axis) in AD subjects when males and females are combined. C) A difference in the slopes of the left olfactory cortical volume with age between controls (grey) and AD (black). D) No difference in the slopes of the right olfactory cortex volume between control and AD with age (x-axis) when males and females are combined.

We next performed a general linear model (GLM) regression analysis in order to determine if age and/or sex impacted on the relationship between OC volume and disease. In the unadjusted model, there was a significant effect of disease on the total (p=0.009) and left (p=0.003) OC volumes. This relationship remained significant when the model was adjusted for age (total OCV, p=0.03; left OCV p=0.012) or sex (total OCV, p=0.015; left OCV, p=0.010; right OCV, p=0.04) (Table 5.1.2). When men and women were analysed separately, the relationship between OCV and disease remained even

when adjusting for age (men: total OCV, p=0.04; left OCV, p=0.014; women, total OCV, p=0.03, left, p=0.02, Table 5.1.2).

Table 5.1.2: Multiple linear regression analysis of olfactory cortical volume and disease.

	OCV total				OCV left				OCV right			
	Variables	**β**	**Std Error**	**p-value**	**Variables**	**β**	**Std Error**	**p-value**	**Variables**	**β**	**Std Error**	**p-value**
Women	**Unadjusted**				**Unadjusted**				**Unadjusted**			
	($F=3.731$, $R^2=0.151$, $p=0.067$)				($F=4.632$, $R^2=0.181$, $p=0.043$*)				($F=1.801$, $R^2=0.079$, $p=0.194$)			
	Disease	-0.388	0.127	0.067	Disease	-0.425	0.08	0.043*	Disease	-0.281	0.055	0.194
	Adjusted for the age				**Adjusted for the age**				**Adjusted for the age**			
	($F=4.015$, $R^2=0.286$, $p=0.034$*)				($F=4.903$, $R^2=0.329$, $p=0.019$*)				($F=1.981$, $R^2=0.165$, $p=0.164$)			
	Disease	-0.205	0.133	0.343	Disease	-0.233	0.082	0.019*	Disease	-0.135	0.06	0.562
	Age	-0.411	0.013	0.065	Age	-0.43	0.008	0.048*	Age	-0.328	0.006	0.166
Men	**Unadjusted**				**Unadjusted**				**Unadjusted**			
	($F=4.825$, $R^2=0.256$, p=0.0,045*)				($F=6.826$, $R^2=0.328$, $p=0.02$*)				($F=2.724$, $R^2=0.163$, $p=0.121$)			
	Disease	-0.506	0.148	0.045*	Disease	-0.573	0.073	0.02*	Disease	-0.404	0.081	0.121
	Adjusted for the age				**Adjusted for the age**				**Adjusted for the age**			
	($F=4.229$, $R^2=0.394$, $p=0.038$*)				($F=5.985$, $R^2=0.479$, $p=0.014$*)				($F=2.397$, $R^2=0.269$, $p=0.130$)			
	Disease	-0.558	0.14	0.024*	Disease	-0.626	0.067	0.008*	Disease	-0.449	0.08	0.083
	Age	0.375	0.012	0.109	Age	0.393	0.006	0.074	Age	0.329	0.007	0.192
Men and women	**Unadjusted**				**Unadjusted**				**Unadjusted**			
	($F=7.669$, $R^2=0.172$, $p=0.009$*)				($F=10.199$, $R^2=0.216$, $p=0.003$*)				($F=3.680$, $R^2=0.090$, $p=0.063$)			
	Disease	-0.414	0.096	0.009*	Disease	-0.465	0.055	0.003*	Disease	-0.301	0.047	0.063
	Adjusted for the age				**Adjusted for the age**				**Adjusted for the age**			
	($F=3.731$, $R^2=0.172$, $p=0.034$*)				($F=5.041$, $R^2=0.219$, $p=0.012$*)				($F=1.895$, $R^2=0.095$, $p=0.165$)			
	Disease	-0.415	0.103	0.014*	Disease	-0.447	0.059	0.007*	Disease	-0.324	0.05	0.061
	Age	0.002	0.01	0.99	Age	-0.055	0.005	0.727	Age	0.073	0.005	0.666
	Adjusted for the sex				**Adjusted for the sex**				**Adjusted for the sex**			
	($F=3.481$, R2=0.209, $p=0.015$*)				($F=5.309$, R2=0.228, $p=0.01$*)				($F=3.622$, R2=0.168, $p=0.037$*)			
	Disease	-0.43	0.095	0.006*	Disease	-0.474	0.055	0.003*	Disease	-0.323	0.046	0.041*
	Sex	0.194	0.093	0.199	Sex	0.108	0.054	0.465	Sex	0.278	0.045	0.076

Age influence on the volume of the hippocampus

In order to determine if ageing influenced hippocampal volume we first performed a Pearson's correlation test. We found no significant correlation between age and the volume of the hippocampus in the control group in the left ($r=0.0463$, $r^2=0.002$, $p=0.826$) or the right ($r=1832$, $r^2=0.033$, $p=0.381$) or between the slopes ($p=0.611$) (Fig. 5.1.4A). A similar result was found in the AD group as there was no significant association between age and the left hippocampus volume ($r=0.0298$, $r^2=0.0009$, $p=0.919$) or the right volume ($r=0.1466$, $r^2=0.021$, $p=0.617$) and no significant differences between the slopes ($p=0.804$). (Fig. 5.1.4B). Furthermore, no significant difference was detected when comparing the slopes of the left ($p=0.986$) or right volumes ($p= 0.917$) and age in the control vs. AD groups (Fig. 5.1.4C, D). We next assessed if age or sex impacted the relationship between total hippocampal volume and disease using a GLM regression analysis. In the unadjusted model, disease had an effect on hippocampal volume as expected ($p=0.03$). When the model was adjusted for age, this significant effect disappeared as the association was no longer significant. However, the disease was still significant ($p=0.03$). When the model was adjusted for sex, including disease-sex interactions, the results demonstrated that disease impacted hippocampal volumes (total hippocampal volume, $p=0.002$). When testing sexes separately, in men, the relationship held when adjusting for age only when assessing the right hippocampal volume ($p=0.03$). In women, no significant relationship was observed between disease and hippocampal volume.

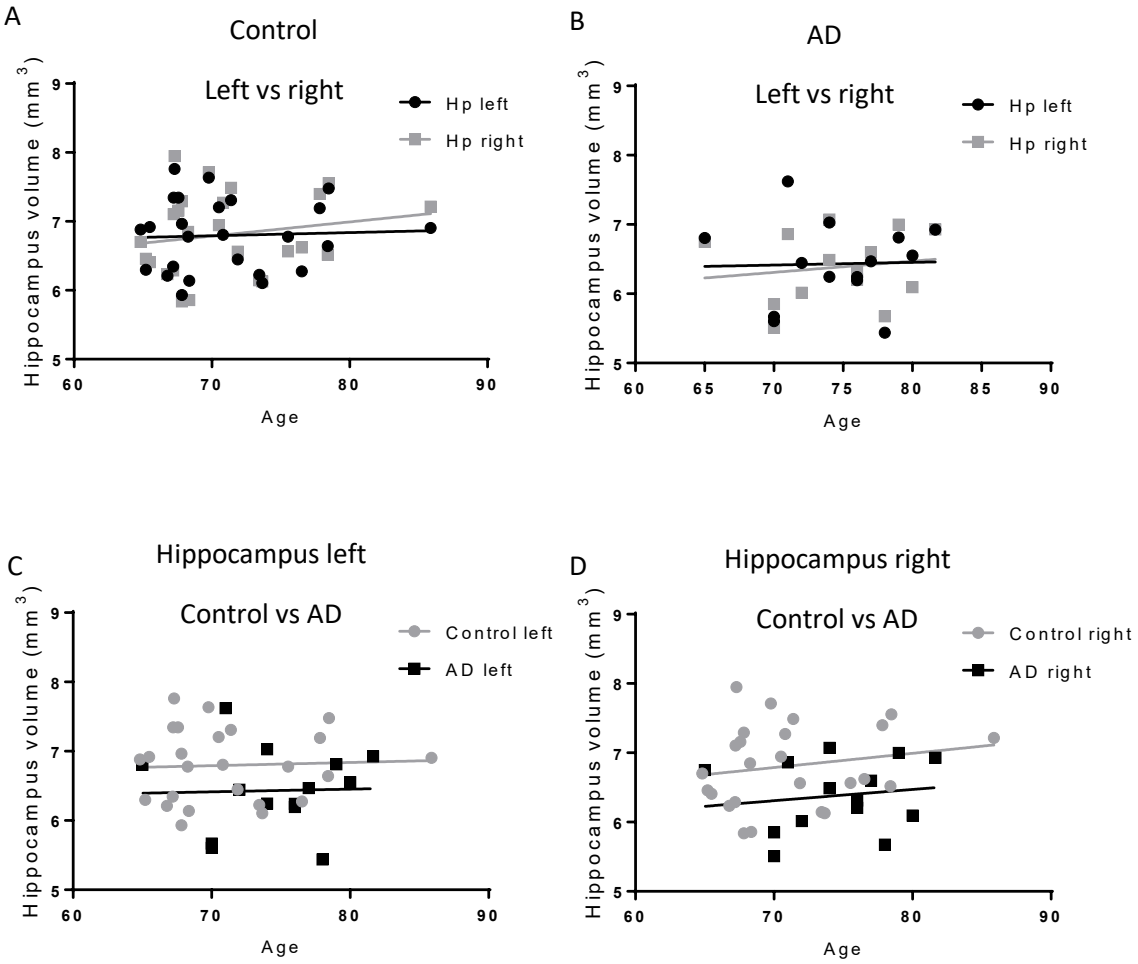


Figure 5.1.4: The relationship between hippocampal volume and age in the AD and control participants. A) No significant correlation in controls between the left hippocampus volume (black) or right (grey) and age (x-axis) when males and females are combined. B) A similar result is seen in AD cases for both left and right hippocampus volume. C) No difference between the slopes of the left hippocampus volume (y-axis) with age (x-axis) in control (grey) compared to AD (black) when males and females are combined. D) Similarly, no difference is detected between the slopes of the right hippocampus volume with age in controls compared with AD.

Levels of education and influence on the volume of the hippocampus in controls

We next assessed whether years of education impacted OC or hippocampal volumes. There was no significant correlation observed between education (in years) and

OC volume in control or AD participants (data not shown). However, there was a positive correlation in the controls (men and women combined) between years of education and total hippocampal volume (Fig. 5.1.5A, r=0.4125, R^2=0.170, p=0.04) and left hippocampal volume (Fig. 5.1.5B, r=0.4132, R^2=0.171, p=0.04), while only a trend was found for the right hippocampal volume (Fig. 5.1.5C, r=0.3955, R^2=0.156, p=0.0504). In the control participants, higher levels of education correlated with hippocampal volume. In sharp contrast, this was not observed in the AD group for the total (r=0.2477, r^2=0.061, p=0.415), left (r=0.2509, r^2=0.062, p=0.408) and right (r=0.2319, r^2=0.0538, p=0.446) hippocampal volume (men and women combined, Fig. 5.1.5A-C).

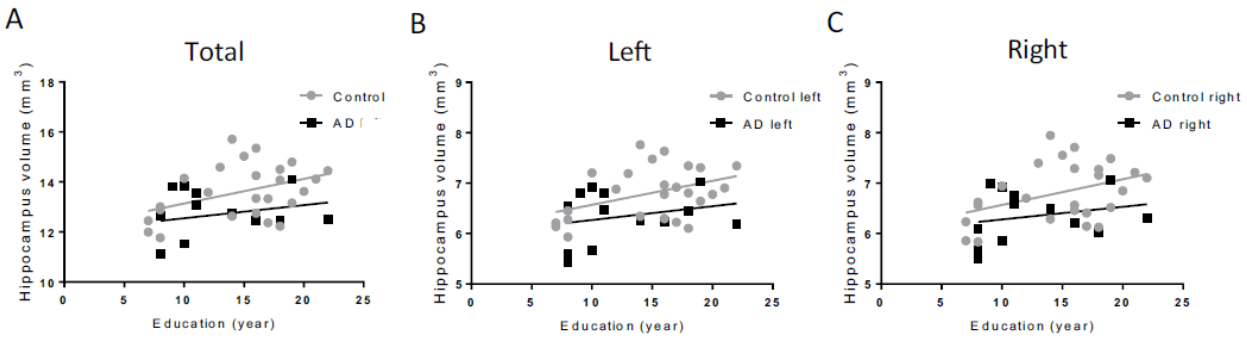


Figure 5.1.5: Graphs showing the correlation between years of education and hippocampal volume in AD and control participants A) A positive correlation between the total volume of the hippocampus and education levels (x-axis) in the control participants (grey). Of note, no such correlation is observed between hippocampus volume (y-axis) and education levels (x-axis) in AD (black). B) A positive correlation between hippocampus left volume (y-axis) and education levels (x-axis) in control participants (grey) that is not found in AD (black). C) No correlation is observed between education levels (x-axis) and the right hippocampus volume in the control group (grey) or in the AD group (black).

5.1.4 Discussion

This study found that the volume of the olfactory cortex was smaller in both men and women with AD when compared with controls. This difference was more apparent in the left olfactory cortex and was influenced by age. As expected, hippocampal volume was also significantly smaller in AD. However, this was only observed in the male cohort. A significant correlation was observed between levels of education and hippocampal volume in controls that was not detected in the AD participants. Asymmetry was observed in the olfactory cortex volume when comparing left and right volumes in both the control and AD participants. This asymmetry in volume loss was not observed in the hippocampus.

The results from the MRI assessment of this cohort demonstrate that both OC and hippocampal atrophy are observed in the AD participants when the sexes are combined. However, when the data are separated by sex, while OC atrophy was observed in both sexes, hippocampal atrophy was only observed in men with AD and not women. In addition, the olfactory cortex showed asymmetry in both the control and AD groups. In contrast, a comparison of the left and right volumes of the hippocampus showed no such asymmetry for either group. GLM regression analysis demonstrated that the predominant effect on the OC volume in the AD participants was due to disease and not age or sex. Although there was a significant association between years of education and the volume of the hippocampus in controls.

Numerous articles have reported that olfactory dysfunction is observed in the majority of AD cases (Kashibayashi et al., 2020; Murphy, 2019; Velayudhan, 2015) and that this is an early symptom of the disease (Doty, 2009; Wilson et al., 2009). Furthermore, a number of publications have shown that atrophy of olfactory brain regions occurs in AD. This includes structures such as the olfactory bulb (Buschhüter et al., 2008, p.; Christen-Zaech et al., 2003; Thomann, et al., 2009), olfactory tract (Thomann, et al., 2009), olfactory epithelium (Arnold et al., 2010) and entorhinal cortex (Devanand et al., 2007; Juottonen et al., 1998). Olfactory dysfunction and olfactory brain region atrophy is also observed in MCI (Quarmley et al., 2016; Thomann, et al., 2009) and evidence appears to suggest that this is progressive (Westervelt et al., 2008). However, no studies have specifically assessed the overall OC volume in AD and few studies have assessed the sexes independently despite the fact that more women are affected with AD than men. Our study demonstrates that OC volume is reduced in AD, and despite the low numbers, when separating by sex we see this effect in both men and women. Furthermore, the left OC is more affected than the right OC in AD. The results of the regression analysis demonstrate that disease is a significant predictor of OC volume.

As expected we observed hippocampal atrophy in the AD cases (Mueller et al., 2010). This was most pronounced in the right hippocampus with only a trend difference in the left hippocampal volume as previously observed (Apostolova et al., 2006; Donix et al., 2013). However, this contrasts with other findings showing greater atrophy in the left hippocampus in AD (Murphy et al., 2003; Pievani et al., 2011; Zhao et al., 2019). This finding could be the result of the association between the right hippocampus volume and

stress (Lindgren et al., 2016), depression (Mathias et al., 2016) and/or other factors that influence hippocampus volume including exercise, age, genetic factors or handedness (all participants involved in our cohort were right handed) (Hibar et al., 2015; Mathias et al., 2016; Toga & Thompson, 2003). Hippocampal volume is also influenced by sex. The reasons for this include hormones and/or hormone therapy, patterns of brain development, psychosocial stress responses, longevity and/or inflammatory reaction differences between men and women (Koss & Frick, 2017; Mancini et al., 2017; Toga & Thompson, 2003). Surprisingly, when our results were analysed by sex we only observed hippocampal atrophy in the male AD cases, despite the number being lower in this group compared with the women. There was a subtle but significant age difference between our women control and AD cases that was not present in our male cohort. However, the female AD cases were slightly older than the controls, a characteristic that in theory should have made the difference more pronounced. It is possible that the women AD cases in our cohort might have been an AD subgroup with a less aggressive progression with limited involvement of the hippocampus (Burke et al., 2019). Protocols for identifying this particular subgroup are still lacking in the clinic. While actual atrophy per se and relative sparing of pathology in the hippocampus is observed in cases of this kind, in general, it has been observed that there is increased neurofibrillary tangles in other regions including the nucleus basalis of Meynert and cortical areas in this clinical phenotype (Al-Shaikh et al., 2020; Murray et al., 2011).

In general, the human brain shows functional (brain networks and activity, neurochemical, behavioural) and structural (brain regions, cytoarchitectural, dendritic

arborisation) asymmetry (Toga & Thompson, 2003). In particular, several brain regions, including the frontal lobe, the occipital pole, overall cortical thickness, grey matter volume in the frontal lobe and the anterior insular cortex exhibit asymmetry. Furthermore, sex-dependent brain asymmetry is also common. Conflicting results have been found for the entorhinal olfactory cortex, with some studies showing asymmetry (Kong et al., 2018) while others do not (Donix et al., 2013). The significant asymmetry observed in the left vs. right OC volumes in our control population could be the result of developmental, environmental, sex, pathological factors or hereditary influences (Toga & Thompson, 2003). Of note, in our control cohort, we observed that both men and women presented with OC asymmetry. Regarding the OC volume asymmetry in the AD cases, a number of studies have observed that left-brain regions are more affected in MCI and/or AD (Barnes et al., 2005; Liang et al., 2018; Loewenstein et al., 1989; Yu et al., 2019; Zhao et al., 2019). While the reasons for this asymmetry are still somewhat unclear, a number of hypotheses have been put forward to explain this, including genetic disease risk factors such as carrying the Apolipoprotein E ε4 (*APOE* ε4) allele. Carriers of the *APOE* ε4 allele(s) may have specific regional differences in the left brain regions (Kelly et al., 2018; Liang et al., 2018; Low et al., 2019), and the evidence suggests that the APOE protein may be involved in brain development and the physiological and/or pathological patterns observed later in life (Donix et al., 2013). When inhaling, inputs from both nostrils remain largely separated up to the primary olfactory cortex (Feng & Zhou, 2019). In line with our results on atrophy in the OC in the AD cases, measuring the distance between a participant's nostril and a peanut butter container, the probable AD patients' left nostril

was significantly less than the right showing that there is left nostril odour detection impairment in AD patients as the distance to the left nostril for detecting an odour is significantly shorter to an olfactory stimulus container in AD compared with controls (Stamps et al., 2013). In contrast to the OC, we did not observe any asymmetry in the hippocampus in the control cohort or in the AD participants. A similar finding was observed when separating the groups by sex. Conflicting results have been observed regarding a/symmetry of the hippocampus in AD cases, with some observing asymmetry (Nobis et al., 2019; Pedraza et al., 2004) and others showing similar findings to ours (Donix et al., 2013; Geroldi, 2000). This might be dependent upon the stage of the disease. A longitudinal study showed that asymmetry in left vs. right hippocampal volumes was present at baseline in AD patients (Barnes et al., 2005). However, this was not detectable in the follow-up scans 15 months later, suggesting that the asymmetry was reduced with disease progress.

Age is the highest risk factor for AD, and indeed our results demonstrate age does impact OC volume in the AD participants. However, it is important to note that, irrespective of age, there is an impact of the disease on the OC volume. There was a significant difference between the slopes and different result when controlling for age in women suggesting that the pathogenesis of AD affects the relationship between the OC volume and age. Ageing affects brain size, vasculature, olfaction and cognition (Peters, 2006). Underlying molecular changes have also been shown, including alterations in neurotransmitters, hormones, neurotrophic factors, and reactive oxygen species, among others (Peters, 2006).

Higher education levels, healthy diets and exercise have been linked to better cognitive functioning later in life (Peters, 2006). Indeed, Noble et al. (2012) demonstrated a dose-dependent effect of education on hippocampal volume in cognitively healthy individuals. In agreement with other studies (Noble et al., 2012), we noted a significant association between education levels and the volume of the hippocampus in the controls. This was not detected in the AD cases despite no significant difference in education levels between the control and AD participants. Reduced levels of education have been linked to increased exposure to life stress, which is associated with hippocampal structural differences (Noble et al., 2012). A number of environmental factors may affect brain development including, poverty, exposure to violence, family turmoil and instability (Evans, 2004; Noble et al., 2012); thus it cannot be excluded that the observed effect might be solely due to number of years of education in our control cohort. However, cognitive reserve has been shown to have clear beneficial effects on brain structure and function and may provide protection against neurodegeneration (Noble et al., 2012; Stern, 2002, 2006).

Our study suggests that the volume of the OC may be used as a disease proxy in AD, thus saving considerable time in the detailed segmentation analysis necessary to obtain volumetric measures of specific olfactory brain regions such as the piriform and/or entorhinal cortex. Additional studies are required to establish a sufficient baseline for the volume of the OC in control and MCI/AD cases in order to validate these data further. Moreover, more research is required to understand the links between the early olfactory dysfunction observed in neurodegenerative diseases including MCI, AD, PD and HD and

how this impacts the structures in the olfactory regions of the brain and *vice versa*. The findings in this study highlight the importance of the role of olfactory cortical atrophy in the pathological course of AD and the interplay between olfactory deficits and degeneration of olfactory regions in the brain.

5.2 Experiment 5: Volumetric changes in olfaction related brain regions in MCI and AD

5.2.1 Introduction

Dementia is a common disease that is difficult to live with, and it affects an individual's cognitive and sensory abilities that are essential to deal with normal daily activities. The most common cause of dementia is Alzheimer's disease (AD) that is a progressive neurodegenerative disease (Nebel et al., 2018). It affects large numbers of elderly people in all societies. AD prevalence is expected to double its number on average within a 20 year lifespan in the developing countries (Kocahan & Doğan, 2017). In the United State, the percentage of AD patients increases with age with a 3% increase observed between the age of 65-74 year, a 17% increase between the age of 75-84 year and a 32% increase between the age of 85 year and above (Hebert et al., 2013). The main AD hallmark includes extracellular senile plaques and intracellular neurofibrillary tangles (Alafuzoff et al., 2008; Yilmaz et al., 2017). It is true that Alzheimer's disease progression takes time and related lesions that accumulate in the brain need a long time to be of burden and start to affect neuropathologically an individual (Perl, 2010). The disease, however, is a serious illness in all its stages. Even if the neuropathology of this disease has been well investigated, the causes of these alterations remain not clearly understood (Nebel et al., 2018). Alzheimer's disease, like many other neurodegenerative diseases, in addition to impairing cognitive abilities also appears to cause olfactory

deficits and there is accumulating evidence that indicates that olfactory dysfunction is an early manifestation of AD (Wilson et al., 2009; Zou et al., 2016).

There is a fine line between healthy cognitive function in the elderly and cognitive decline. To establish whether a person is experiencing cognitive decline in the clinic, evidence of deterioration in performance in one or more cognitive domains of a magnitude greater than expected for a patient's age and educational background should be documented (Albert et al., 2011). However, it is difficult to establish a clear demarcation between physiological age-related cognitive decline and MCI; in this latter case an important role is played by clinical judgment that can guide diagnosis (Albert et al., 2011). Once the presence of mild cognitive impairment is established, approximately 10% of cases with MCI progress to probable AD dementia within a year (Mitchell & Shiri-Feshki, 2009; Yilmaz et al., 2017). Another study reported that 68% of patients with MCI progress to AD dementia at a 28 month follow up (Mitolo et al., 2019). While a combination of cognitive tests helps to predict progression from MCI to dementia, some studies considered neuroimaging measures as good predictor tools of future cognitive decline and others found these to be excellent predictors (Belleville et al., 2014).

Neuroimaging measurements such as volumetric measures are useful predictors independently of other biomarkers including being a carrier of the Apolipoprotein E ε4 allele (Petersen, 2004). In clinical studies, hippocampus, entorhinal cortex and whole brain volumetric measurements have been commonly used as predictors of progression from MCI to AD dementia using structural MRI (Petersen et al., 2009). It is also well known

that cognitive abilities decline with age and age is also associated with change in brain volume and function (Dennis & Thompson, 2014). Olfactory impairment was found to be present in MCI and to be associated with greater decline in cognitive performance at follow up (Roberts et al., 2016). Olfactory impairment is further increased in the presence of neurodegenerative disorders such as Alzheimer's disease and olfactory dysfunction may be considered an early marker of cognitive decline (Larsson et al., 2016). In fact, there is evidence that MCI patients who progress to AD dementia have greater grey matter volume reduction than stable MCI patients (Franks et al., 2015). It has also been reported that the degree of atrophy of the olfactory cortex (especially the entorhinal cortex) may predict progression from MCI to AD dementia and could help in the early diagnosis of AD (Stoub et al., 2005; Traschütz et al., 2020).

Typical Alzheimer's disease hallmarks (neurofibrillary tangles and senile plaques) are found in all cortical areas; neurofibrillary tangles are found in greater quantities in the medial temporal lobe including the hippocampus, parahippocampus, amygdala and entorhinal cortex, first in the entorhinal cortex, hippocampus and then in the amygdala (Braak et al., 1996; McDonald & Mott, 2017). The brain alteration due to AD occur in different regions in the brain as the literature points out. A study indicated that the pathological brain changes in AD patients begin in the hippocampus, parahippocampus, amygdala and entorhinal cortex (Iizuka et al., 2021). A more recent study has indicated that early AD pathology damages the olfactory cortex and hippocampus first (Lu et al., 2019). In terms of brain atrophy, age influences the extent of brain loss in AD patients and the effects are different from those observed as a result of normal ageing (Fiford et

al., 2018). Younger AD patients have more brain and hippocampal atrophy as well as overall brain volume loss than older AD patients (Fiford et al., 2018). AD related olfactory dysfunction might be caused by the disruption in the olfactory network and its connection (Lu et al., 2019).

For sexes, women show a faster rate of hippocampal atrophy than men in the case of AD (Mazure & Swendsen, 2016) and MCI (Sundermann et al., 2017). Greater hippocampal atrophy rate with increasing age has been found in the control population (Fiford et al., 2018), but in AD patients there appears to be less severe atrophy rate with more advanced age (Fiford et al., 2018; Holland et al., 2013). The left hippocampal volume is an effective indicator of the AD stage (Uysal & Ozturk, 2020). It has been found that volume reduction of the left hippocampus is associated with the memory and cognitive decline observed in AD (Peng et al., 2015). On the other hand, right hippocampal atrophy is associated with a more advanced stage of AD (Lee et al., 2019). NFT are found mainly in hippocampal regions in the AD brain (Sengoku, 2020). Moreover, the accumulation of Aβ and Tau has been found in the olfactory cortex, orbitofrontal cortex, entorhinal cortex and hippocampus and associated with olfactory memory discrimination in AD mice (Cassano et al., 2011). The amygdala (especially the right volume), parahippocampus and hippocampus volumes show moderate association with odour threshold and the left hippocampus volume particularly shows a strong association with the scores achieved by AD patients on the olfactory identification test (Murphy et al., 2003). Also, using the UPSIT test, an fMRI study found that the signal of blood oxygen level dependent was reduced in the olfactory cortex and hippocampus in AD patients

compared with healthy controls (Wang et al., 2010). Moreover, an association was found between atrophy of the olfactory bulb and tract, and medial temporal lobe density in AD patients (Thomann, et al., 2009).

Understanding and measuring olfactory-related brain regions is important since these are involved in the most common neurodegenerative disease that is Alzheimer's disease, a condition that has olfactory deficit as risk factor.

Aim and Hypothesis:

Aim:

In this study, the aim was to measure the volume of neural regions of the olfactory system in a large number of participants using high resolution three-dimensional structural MRI scans. Measurements of olfactory related brain regions were carried out in three age matched groups, namely neurologically healthy elderly, MCI patients and patients with mild probable AD dementia. The chosen olfactory related brain regions of interest are the olfactory cortex, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex. In addition, volumes of olfactory-related brain regions were also compared in a sub-group of patients for whom the *APOE* ε4 genotype was known.

Hypothesis:

We hypothesised that volumes of olfactory related brain regions will be smaller in patients than in healthy age matched controls with greater differences observed in

patients with mild probable AD dementia and more modest differences seen in the MCI group. Also, there would be volume reduction differences between females and males. In addition, it was hypothesised that *APOE* ε4 carriers would have smaller volumes in olfactory related brain regions than *APOE* ε4 non-carriers.

5.2.2 Methods and materials:

Participants:

Neurologically healthy participants and patients were selected from the datasets part of the Sheffield Ageing Database, an initiative coordinated by the University of Sheffield Department of Neuroscience. The datasets used in this study were from participants recruited between June 2011 and July 2016. The participants had an age range between 55 and 88 years with a mean of 73.86 year and standard deviation of 5.78. A total of two hundred thirteen participants were selected for the purpose of this retrospective study. These included 95 datasets of cognitively healthy participants, 81 datasets of MCI patients and 37 datasets of patients with mild probable AD dementia. Only participants who had a threedimensional structural brain MRI scan were selected. Each dataset was inspected for quality control and depending on their diagnostic status assigned to three groups: “healthy control”, i.e., aged 66-85 (n = 95), “MCI patients”, i.e., aged 58-86 (n = 81) and “AD patient”, i.e., aged 55-88 (n = 37). Clinical profiling via comprehensive neuropsychological testing was carried out for all participants who were

included in this study to confirm their cognitive and clinical status. This assessment battery included the Mini-Mental State Examination (MMSE) and test of short-term and working memory, episodic memory, lexical-semantic processing, attentive-executive functions and visuoconstructive abilities (De Marco et al., 2017, 2019). Eligible participants in the control group were those with no neurological and cognitive decline based on their scores on different cognitive tests being within normal age range. Participants with mild cognitive impairment were diagnosed using Petersen's diagnostic criteria (Petersen, 2004) and AD mild dementia patients were diagnosed using McKhann et al's clinical diagnostic criteria (McKhann et al., 2011). All participants completed a 1.5 T MRI protocol inclusive of a T1-weighted brain scan and biological samples were obtained to identify *APOE* ε4 status, an AD-related risk gene. There were a total of 59 patients for whom *APOE* status was available and these included 22 *APOE* ε4 carriers 37 *APOE* ε4 non-carriers. Despite the large size of the cohort in the database, however, not all participants had agreed to provide a biological sample at the time of recruitment. Additionally, demographic variables were used in the analyses including sex and years of education, given the relationship found between education and brain regional volume (Mortby et al., 2014).

All participants provided written consent. Ethical approval for these retrospective data analyses was obtained from the West of Scotland Regional Ethics Committee 5, with Reference number: 19/WS/0177. All procedures at the basis of the participants' clinical

assessments for this study followed the institutional ethical standards and were in compliance with the Helsinki declaration.

MRI acquisition:

Each participant had a three-dimensional T1-weighted brain scan acquired as part of a more extensive MRI protocol. A Turbo Field Echo T1-weighted sequence was acquired on a Philips Achieva 1.5 T scanner. Acquisition paramenters are the same as the ones detailed in chapter 4, experiment 3.

MRI processing:

The pre-processing of MRI images was done using the most updated version of the Statistical Parametric Mapping software package (SPM), i.e., version 12 (Wellcome Centre for Human Neuroimaging, London, UK), running in a Matlab R2016b environment, version 9.1 (Mathworks Inc, Natick, Massachusetts, USA). SPM was used to preprocess and segment each T1-weighted image into tissue maps. The resulting tissue maps were grey matter, white matter, cerebrospinal fluid. Of these, the neural tissue maps (grey matter and white matter) and the map of cerebrospinal fluid were included in this study in order to test the study hypothesis. The procedure is the same as the one detailed in chapter 4, experiment 3.

Extraction of Olfactory Regions Volumes:

In order to extract and calculate individual total intracranial volumes, tissue-class volumes were individually extracted in SPM12 using the individual's images containing the segmentation parameter (Malone et al., 2015). Grey matter volume, white matter volume and CSF volume were computed in litres and converted to millilitres to be compatible with the unit of measurement of olfactory brain regions' volumes during data analysis. Summing the volume of the three sub-maps was carried out to calculate the individual Total Intracranial Volume (TIV). The methodology detailed in chapter 4, experiment 3 was also used here to extract the olfactory related brain regions of interest.

Data analysis:

Statistical data analysis was performed using the SPSS software 26 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) and the latest version of GraphPad Prism 9 (GraphPad Software San Diego, CA, USA). Descriptive statistics were run to characterise the study population as shown in (table 5.2.1). To explore the data and answer the study question, a one-way ANOVA was the first model run to compare the brain regions' volume among the three groups of neurologically healthy, MCI and AD participants. To fulfil the purpose of the study investigation, analyses of covariance (ANCOVA) were used in the form of a Univariate General Linear Model to regress out the influence of education and TIV. Shapiro-Wilk and Kolmogorov-Smirnov test significance level along with the inspection of the histograms illustrating the frequency distribution of

outcome variables were considered as part of the normality diagnostics, to verify if the volumes were normally distributed. Pearson's correlation coefficient was used to investigate the association between age, education and TIV, and each of the volumetric measures. In order to determine the influence of the *APOE* ε4 allele risk factor on the volume of olfactory regions, t tests were carried out between the small sample of *APOE* ε4 carriers and the group of non-carriers. The statistical threshold to define the significance level for the ROI volume comparison was set to $p < 0.001$.

All contrasts were devised to test our hypotheses. The olfactory related brain regions selected in this analysis include the olfactory cortex, hippocampus, parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex. The volume of these regions was extracted for the purpose of this study.

5.2.3 Result:

All ROI volumes were normally distributed while TIV was not normally distributed. This study populations were age matched as no significant difference was found between the three groups in age (both sexes p=0.33, females p=0.09 and males p=0.36). However, significant differences among the groups were found for education (both sexes p=0.001, females p=0.02 and males p=0.01) and MMSE (both sexes p=0.0001, females p=<0.0001 and males p=<0.0001) (Table 5.2.1). In detail, in both females and males, elderly controls had significantly higher education level than the AD patients (p=0.02, p=0.01

respectively). For MMSE, post-hoc comparisons showed significant differences for both females and males between elderly controls and MCI patients, between elderly controls and AD patients, and between MCI patients and AD patients (F: p=0.01, p<0.0001 and p<0.0001) and (M: p=0.01, p<0.0001 and p<0.0001) respectively.

Table 5.2.1: Demographic Characteristics of the participants

	Female				Male			
	N	Mean (SD)			N	Mean (SD)		
		Age	Education	MMSE		Age	Education	MMSE
Elderly	57	72.4(4.3)	10.3(3.9)	28.5(1.7)	38	73.4(4.9)	12.7(4.8)	28.4(1.4)
MCI	47	73.9(6.8)	8.9(3.2)	27.4(1.9)	34	74.9(5.3)	11.5(4.2)	27(1.9)
AD	19	75.7(7.7)	7.6(3.6)	20.7(2.1)	18	75.3(6.8)	8.6(4.5)	21.2(2.7)
P value		0.09	0.02	<0.0001		0.36	0.01	<0.0001

(P ≤0.05).

One-way ANOVA analyses showed significant differences in volume among the three groups in all olfaction-related brain regions for total volume, and left and right volume separately in men and women (p≤0.0001). *Post-hoc* multiple comparison Bonferroni tests showed significant differences between group pairs. In detail, there was a significant difference between elderly participants and the probable AD dementia patients in all ROIs volumes. For the MCI groups, significant differences from elderly controls and probable AD dementia varied depending on the regions, hemisphere and sex. A similarity in the pattern of volume reduction was observed in the olfactory cortex

and in the orbitofrontal cortex in the MCI group. Moreover, when split by sex, a similar finding was observed for the hippocampus, parahippocampus and amygdala volumes. However, these regions showed significant differences in the MCI group volume when both hemispheres were combined in a total regional volume. In the MCI group the entorhinal cortex volume showed a different pattern of volumetric reduction. ANCOVA analysis showed no change in significant differences in all olfaction-related brain regions when controlling for education or TIV except for MMSE score where significant differences were no longer detected. No association was found between age, education or MMSE with TIV when both sexes were combined or separated for the AD dementia group while education was found to be associated with TIV in the MCI group (Table 5.2.2).

Table 5.2.2: Association of age, education and MMSE with TIV.

TIV	Age		Education		MMSE	
	R^2	p value	R^2	p value	R^2	p value
Elderly						
Both sexes	0.015	0.244	0.048	0.034	0.038	0.06
Women	0.096	0.0.018	0.012	0.45	0.039	0.14
Men	0.029	0.303	0.075	0.1	0.112	0.04
MCI						
Both sexes	0.031	0.12	0.209	0.0001	0.004	0.57
Women	0.083	0.05	0.107	0.03	0.019	0.92
Men	0.002	0.82	0.151	0.023	0.0004	0.91
AD						
Both sexes	0.029	0.92	0.082	0.09	0.007	0.62
Women	0.023	0.54	0.082	0.23	0.0003	0.95
Men	0.006	0.76	0.081	0.25	0.003	0.83

($p \leq 0.05$).

Olfactory cortex volume:

One-way ANOVA analyses showed significant differences in volume between the three groups in the OC (F=21.18, DF=(2,210), p≤0.0001) (figure 5.2.1). *Post-hoc* multiple comparison Bonferroni tests showed that there were significant differences in the volume of this structure for women and men separately in the probable AD dementia group when compared with the elderly controls and for men only when compared with the MCI group (Figure 5.2.1, table 5.2.3 for females and table 5.2.4 for males).

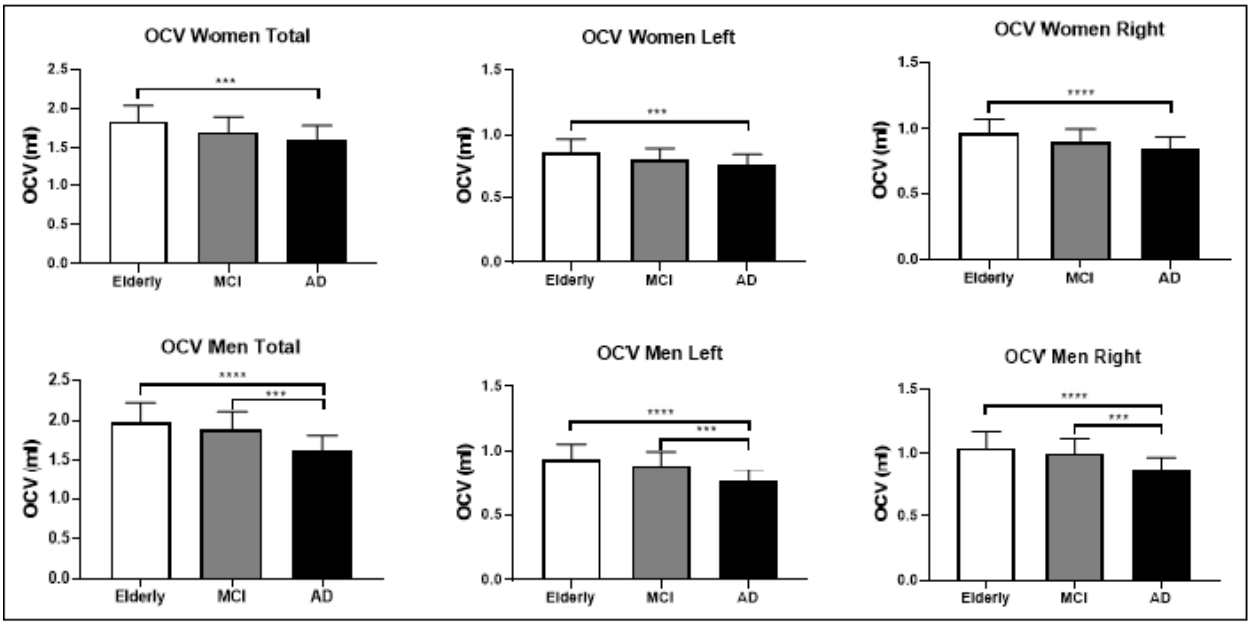


Figure 5.2.1: olfactory cortex volume differences among the three groups: comparisons among the three groups are shown for the female population (above) and male population (below) separately.

The MCI group had a significantly bigger volume than the probable AD dementia patients in men only for the total, left and right volume. Pearson correlation analysis showed no significant association between the volume of the OC and age except for men in both the MCI and probable AD dementia patients. The association was negative

(p=0.04 for both groups) (table 5.2.5, 5.2.6 and 5.2.7). Education was found to be positively associated with the volume of the OC only for men in the MCI group (p=0.01) (table 5.2.6). A positive association was found between MMSE scores and volume of the OC in the elderly female group only (p=0.01) (table 5.2.5). Finally, TIV was found to be positively associated with the volume of the OC in all three groups (elderly, MCI and AD) in women and men, except for men in the AD dementia group (table 5.2.5, 5.2.6 and 5.2.7). When an ANCOVA was carried out, the findings showed that among groups significant differences remained after covarying for each of these variables: age (Women F=9.979, DF=(2,119), p= 0.0001, Men F=14.445, DF=(2,86), p= 0.0001), education (Women F=9.853, DF=(2,119), p= 0.0001, Men F=10.451, DF=(2,86), p= 0.0001) or TIV (Women F=9.039, DF=(2,119), p=0.0001, Men F=20.786, DF=(2,86), p=0.0001). When adjusting for MMSE, the differences among the three groups were no longer significant in the female and male population in OCV (Women F=4.223, DF=(2,119), p= 0.017, Men F=1.918, DF=(2,86), p= 0.153) and all pairwise comparisons showed no significant differences in the comparisons of elderly with MCI, elderly with AD dementia and MCI with AD dementia. MMSE accounted for the differences in OC volume when comparing the three groups, elderly, MCI and AD dementia.

Table 5.2.3: Significant difference in volume in the olfactory regions observed in female groups.

	Female	Elderly vs. MCI Mean Diff. (p Value)	Elderly vs. AD Mean Diff. (p Value)	MCI vs. AD Mean Diff. (p Value)			Elderly vs. MCI Mean Diff. (p Value)	Elderly vs. AD Mean Diff. (p Value)	MCI vs. AD Mean Diff. (p Value)
Olfactory Cortex	Total F=11.5 (P=<0.0001)	0.13 (0.002)	0.22 (0.0001)	0.08 (0.31)	Amygdala	Total F=22.9 (P=<0.0001)	0.11 (0.0005)	0.25 (<0.0001)	0.14 (0.002)
	Left F=10.7 (P=<0.0001)	0.06 (0.003)	0.11 (0.0002)	0.04 (0.37)		Left F=25.05 (P=<0.0001)	0.1 (0.0006)	0.14 (<0.0001)	0.1 (0.0004)
	Right F=11.7 (P=<0.0001)	0.07 (0.002)	0.12 (<0.0001)	0.05 (0.27)		Right F=17.5 (P=<0.0001)	0.05 (0.0014)	0.11 (<0.0001)	0.1 (0.014)
Hippocampus	Total F=28.2 (P=<0.0001)	0.47 (<0.0001)	1.05 (<0.0001)	0.57 (0.0006)	Orbitofrontal cortex	Total F=18.3 (P=<0.0001)	1.18 (0.002)	2.6 (<0.0001)	1.5 (0.01)
	Left F=30.9 (P=<0.0001)	0.27 (<0.0001)	0.62 (<0.0001)	0.35 (0.0002)		Left F=20.1 (P=<0.0001)	0.6 (0.002)	1.4 (<0.0001)	0.81 (0.002)
	Right F=18.4 (P=<0.0001)	0.21 (0.001)	0.42 (<0.0001)	0.22 (0.011)		Right F=15.6 (P=<0.0001)	0.6 (0.002)	1.2 (<0.0001)	0.64 (0.03)
Parahippocampus	Total F=22.1 (P=<0.0001)	0.42 (0.0006)	0.96 (<0.0001)	0.53 (0.002)	Entorhinal Cortex	Total F=25.7 (P=<0.0001)	0.15 (0.0001)	0.34 (<0.0001)	0.19 (0.001)
	Left F=25.7 (P=<0.0001)	0.2 (0.0004)	0.48 (<0.0001)	0.28 (0.0004)		Left F=25.6 (P=<0.0001)	0.08 (0.0002)	0.17 (<0.0001)	0.1 (0.0008)
	Right F=17.1 (P=<0.0001)	0.22 (0.014)	0.47 (<0.0001)	0.25 (0.014)		Right F=22.05 (P=<0.0001)	0.1 (0.0003)	0.2 (<0.0001)	0.1 (0.004)

(Threshold p<0.001).

Table 5.2.4: Significant difference in volume in the olfactory regions observed in male groups.

	Male	Elderly vs. MCI Mean Diff. (p Value)	Elderly vs. AD Mean Diff. (p Value)	MCI vs. AD Mean Diff. (p Value)			Elderly vs. MCI Mean Diff. (p Value)	Elderly vs. AD Mean Diff. (p Value)	MCI vs. AD Mean Diff. (p Value)
Olfactory Cortex	Total F=15.3 (P=<0.0001)	0.09 (0.32)	0.36 (<0.0001)	0.27 (0.0003)	Amygdala	Total F=18.6 (P=<0.0001)	0.07 (0.33)	0.3 (<0.0001)	0.24 (<0.0001)
	Left F=15.9 (P=<0.0001)	0.05 (0.25)	0.18 (<0.0001)	0.13 (0.0003)		Left F=22.2 (P=<0.0001)	0.04 (0.29)	0.2 (<0.0001)	0.14 (<0.0001)
	Right F=14.04 (P=<0.0001)	0.04 (0.41)	0.18 (<0.0001)	0.14 (0.0005)		Right F=12.4 (P=<0.0001)	0.03 (0.5)	0.13 (<0.0001)	0.1 (0.001)
Hippocampus	Total F=20.3 (P=<0.0001)	0.38 (0.072)	1.3 (<0.0001)	0.9 (<0.0001)	Orbitofrontal cortex	Total F=18.1 (P=<0.0001)	0.9 (0.22)	3.6 (<0.0001)	2.7 (<0.0001)
	Left F=26.6 (P=<0.0001)	0.22 (0.04)	0.78 (<0.0001)	0.56 (<0.0001)		Left F=21.9 (P=<0.0001)	0.5 (0.13)	1.9 (<0.0001)	1.5 (<0.0001)
	Right F=11.2 (P=<0.0001)	0.17 (0.2)	0.5 (<0.0001)	0.34 (0.007)		Right F=14 (P=<0.0001)	0.4 (0.4)	1.7 (<0.0001)	1.2 (0.0005)
Parahippocampus	Total F=12.9 (P=<0.0001)	0.32 (0.25)	1.13 (<0.0001)	0.81 (0.002)	Entorhinal Cortex	Total F=14.3 (P=<0.0001)	0.11 (0.14)	0.4 (<0.0001)	0.3 (0.002)
	Left F=16.6 (P=<0.0001)	0.16 (0.19)	0.58 (<0.0001)	0.42 (0.0003)		Left F=17.1 (P=<0.0001)	0.1 (0.11)	0.2 (<0.0001)	0.14 (0.0004)
	Right F=9.4 (P=0.0002)	0.17 (0.34)	0.55 (0.0001)	0.38 (.011)		Right F=10.6 (P=<0.0001)	0.05 (0.23)	0.17 (<0.0001)	0.11 (0.01)

(Threshold p<0.001).

Hippocampus Volume:

One-way ANOVA analyses showed significant differences in volume among the three groups in hippocampus volume (and F=38.26, DF=(2,210), p≤0.0001) (figure 5.2.2). *Post-hoc* multiple comparison Bonferroni tests showed significant differences between groups. However, the right hippocampus volume appeared to show less reduction in volume than the left until a later stage of disease, as significant differences emerged only in the AD dementia versus elderly comparisons in both sexes. For the left volume, MCI were more affected in the left volume in both sexes (Figure 5.2.2, table 5.2.3 for females and table 5.2.4 for males).

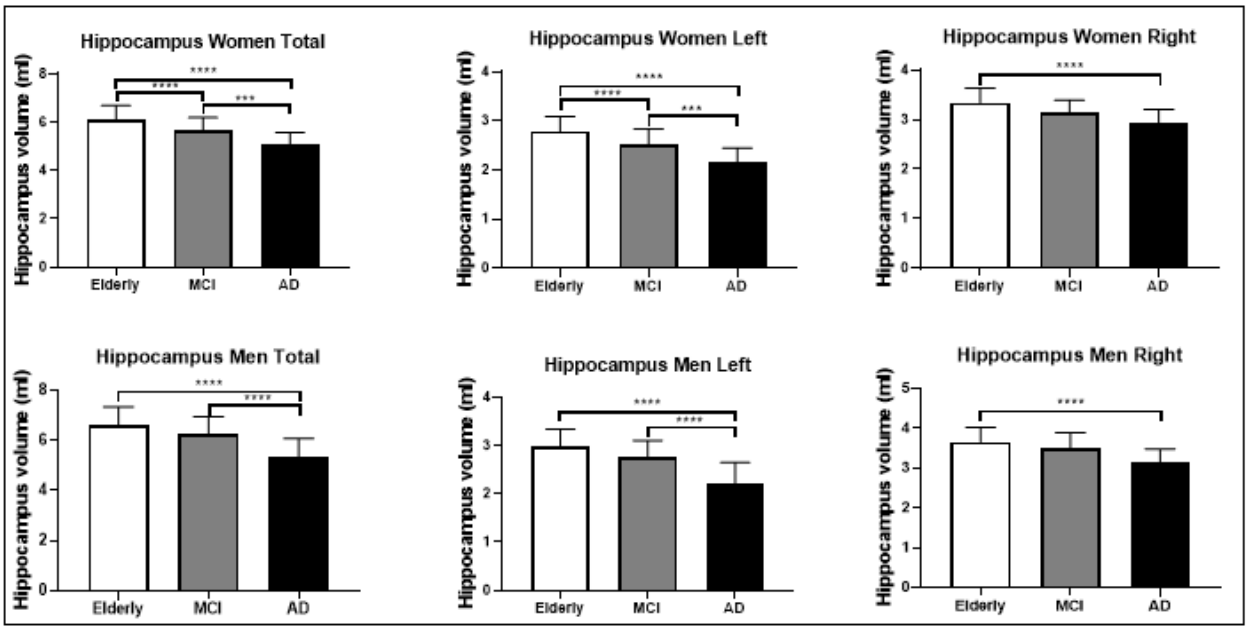


Figure 5.2.2: Hippocampus volume differences among the three groups: comparisons among the three groups are shown for the female population (above) and male population (below) separately.

In women MCI, the total volume and the left hemisphere volume were significantly smaller when compared with the healthy control group and significantly bigger than the probable AD dementia group while in men, the MCI group volume was significantly bigger than the probable AD dementia group for total volume and for the left hemisphere. Correlation analyses showed a significant negative association between hippocampus volume and age in the elderly men group; a positive association was found between MMSE and hippocampus volume in women and in both sexes between hippocampus volume and TIV (table 5.2.5). In women, hippocampus volume in the MCI group was negatively associated with age while positively associated with MMSE (Table 5.2.6). In the probable AD dementia group, a positive association was found between TIV and hippocampus volume in women (table 5.2.7). An ANCOVA showed no significant influence when controlling for age (Women $F=24.742$, $DF=(2,119)$ $p= 0.0001$, Men $F=19.4580$, $DF=(2,86)$, $p=0.0001$), education (Women $F=26.150$, $DF=(2,119)$, $p= 0.0001$, Men $F=16.028$, $DF=(2,86)$, $p=0.0001$) or TIV (Women $F=25.028$, $DF=(2,119)$, $p=0.0001$, Men $F=22.36$, $DF=(2,86)$, $p=0.0001$). When adjusting for MMSE, the difference among the three groups was no longer significant in the female and male population (Women $F=6.356$, $DF=(2,119)$, $p= 0.002$, Men $F=2.274$, $DF=(2,86)$, $p= 0.11$). Similarly no significant differences were found when pairwise comparisons were carried out among the three groups.

Table 5.2.5: Associations between age, education, MMSE scores and TIV with volumes of olfactory-related brain regions in the elderly control group.

Elderly		Age		Education		MMSE		TIV	
	Sex	R square	P value	R square	P value	R square	P value	R square	P value
OCV	Women	0.02	0.28	0.01	0.49	0.121	0.01	0.285	0.0001
	Men	0.08	0.09	0.04	0.21	0.078	0.09	0.583	0.0001
Hippocampus	Women	0.03	0.19	0.02	0.33	0.098	0.02	0.271	0.0001
	Men	0.13	0.03	0.015	0.47	0.064	0.12	0.425	0.0001
Parahippocampus	Women	0.02	0.23	0.015	0.37	0.01	0.02	0.362	0.0001
	Men	0.078	0.09	0.002	0.80	0.062	0.13	0.49	0.0001
Amygdala	Women	0.013	0.39	0.01	0.46	0.135	0.01	0.391	0.0001
	Men	0.116	0.04	0.03	0.31	0.045	0.19	0.546	0.0001
OFC	Women	0.055	0.08	0.004	0.63	0.152	0.003	0.251	0.0001
	Men	0.14	0.02	0.04	0.23	0.054	0.158	0.504	0.0001
Entorhinal Cortex	Women	0.05	0.09	0.01	0.49	0.12	0.01	0.254	0.0001
	Men	0.13	0.03	0.02	0.46	0.05	0.18	0.388	0.0001

($P \leq 0.05$).

Table 5.2.6: Associations between age, education, MMSE scores and TIV with volumes of olfactory-related brain regions in the MCI group.

MCI		Age		Education		MMSE		TIV	
	Sex	R square	P value	R square	P value	R square	P value	R square	P value
OCV	Women	0.01	0.54	0.053	0.12	0.042	0.16	0.305	0.0001
	Men	0.123	0.04	0.179	0.01	0.01	0.62	0.277	0.001
Hippocampus	Women	0.084	0.047	0.001	0.87	0.151	0.01	0.063	0.09
	Men	0.11	0.053	0.109	0.06	0.003	0.76	0.092	0.08
Parahippocampus	Women	0.01	0.61	0.023	0.30	0.093	0.04	0.227	0.001
	Men	0.081	.010	0.096	0.07	0.003	0.74	0.145	0.03
Amygdala	Women	0.001	0.87	0.004	0.67	0.059	0.09	0.300	0.0001
	Men	0.031	0.32	0.174	0.01	0.018	0.44	0.113	0.051
OFC	Women	0.021	o.33	0.103	0.03	0.103	0.03	0.336	0.0001
	Men	0.098	0.07	0.169	0.02	0.005	0.69	0.194	0.01
Entorhinal Cortex	Women	0.035	0.20	0.001	0.82	0.109	0.02	0.150	0.01
	Men	0.1049	0.06	0.121	0.04	0.001	0.89	0.077	0.11

($P \leq 0.05$).

Table 5.2.7: Associations between age, education, MMSE scores and TIV with volumes of olfactory-related brain regions in the probable AD dementia group.

AD		Age		Education		MMSE		TIV	
	Sex	R square	P value	R square	P value	R square	P value	R square	P value
OCV	Women	0.03	0.5	0.085	0.22	0.01	0.69	0.514	0.001
	Men	0.233	0.04	0.031	0.49	0.024	0.53	0.106	0.187
Hippocampus	Women	0.032	0.45	0.198	0.06	0.0001	0.97	0.245	0.03
	Men	0.14	0.12	0.037	0.44	0.186	0.07	0.003	0.83
Parahippocampus	Women	0.004	0.79	0.187	0.06	0.023	0.53	0.419	0.003
	Men	0.074	0.27	0.012	0.66	0.151	0.11	0.042	0.41
Amygdala	Women	0.01	0.69	0.183	0.07	0.001	0.90	0.451	0.002
	Men	0.119	0.16	0.016	0.62	0.276	0.03	0.053	0.35
OFC	Women	0.016	0.59	0.234	0.04	0.0005	0.93	0.499	0.001
	Men	0.168	0.09	0.004	0.79	0.1005	0.20	0.091	0.22
Entorhinal Cortex	Women	0.037	0.43	0.119	0.15	0.003	0.83	0.289	0.02
	Men	0.112	0.17	0.015	0.63	0.206	0.06	0.01	0.75

($P \leq 0.05$).

Parahippocampus Volume:

Analyses showed significant differences in parahippocampus volume among the groups (F=24.63, DF=(2,210), p≤0.0001) (figure 5.2.3). Bonferroni tests showed a significantly smaller parahippocampus volume in the probable AD dementia patients when compared with elderly participants. Also, the difference was significant when comparing probable AD dementia patients with the MCI group in the left hemisphere only for both females and males. Moreover, the total and the left volume was significantly smaller in the female MCI group in comparison with the elderly female participants (Figure 5.2.3, table 5.2.3 for females and table 5.2.4 for males). In the elderly group, a significant positive correlation was found between parahippocampus volume and MMSE scores in women and with TIV in both sexes in the elderly group (Table 5.2.5). For the MCI group, a positive association was found between parahippocampus volume and MMSE in women and between the volume of this region and TIV in both sexes (Table 5.2.6). In the AD group, a positive association was found between parahippocampus volume and TIV only in female patients (table 5.2.7). ANCOVA test showed no significant effect of age (Women F=19.972, DF=(2,119), p= 0.0001, Men F=11.914, DF=(2,86), p=0.0001), education (Women F=19.555, DF=(2,119), p= 0.0001, Men F=10.046, DF=(2,86), p=0.0001) or TIV (Women F=20.391, DF=(2,119), p=0.0001, Men F=15.098, DF=(2,86), p=0.0001). When adjusting for MMSE, the difference among the three groups was no longer significant in the female and male population (Women F=5.053, DF=(2,119), p= 0.008, Men F=1.711, DF=(2,86), p= 0.187). Likewise no significant differences were detected by pairwise comparisons among groups.

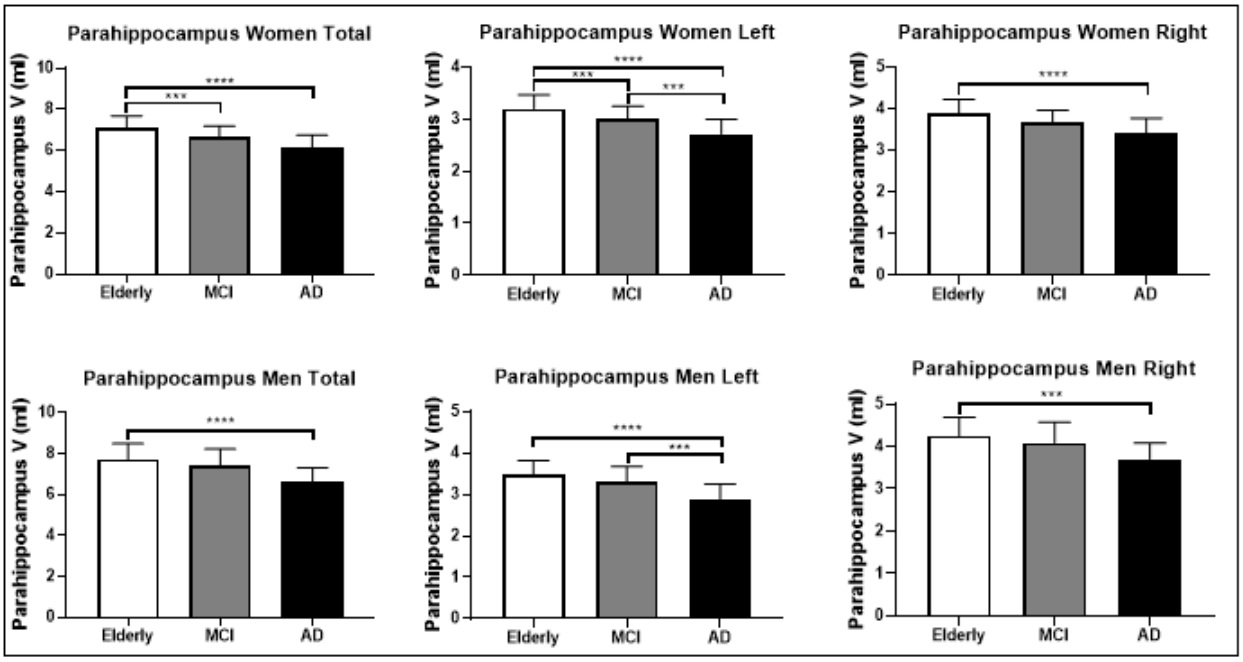


Figure 5.2.3: Parahippocampus volume differences among the three groups: comparisons among the three groups are shown for the female population (above) and male population (below) separately.

Amygdala Volume:

One-way ANOVA analyses showed significant differences in volume among the elderly, MCI and probable AD dementia groups in amygdala volume (F=28.12, DF=(2,210), p≤0.0001) (figure 5.2.4). *Post-hoc* multiple comparison Bonferroni tests showed a significant difference between elderly and probable AD dementia patients in the female and male populations in both hemispheres. Moreover, the volume of the amygdala was significantly bigger in the MCI group when compared with the probable AD dementia group in both sexes in the left hemisphere and for total volume in men. Also, the volume of this structure in the MCI group was significantly smaller than the elderly

female control participants in total and left volume (Figure 5.2.4, table 5.2.3 for females and table 5.2.4 for males). In the elderly population, a significant negative association was found between amygdala volume and age in both sexes: there also was a significant positive association with MMSE in women only, and one with TIV in both sexes (table 5.2.5). In the MCI group, a significant positive correlation was found only with education in the male group and with TIV in the female group (table 5.2.6). In the probable AD dementia group, a significant positive association was found with MMSE in the male group and with TIV in the female group (table 5.2.7). Using ANCOVA, when adjusting for age (Women F=20.895, DF=(2,119), p= 0.0001, Men F=17.701, DF=(2,86), p=0.0001), education (Women F=20.477, DF=(2,119), p=0.0001, Men F=13.8975, DF=(2,86), p=0.0001) or TIV (Women F=22.141, DF=(2,119), p=0.0001, Men F=21.372, DF=(2,86), p=0.0001), significant differences persisted. However, when controlling for MMSE, differences among the three groups were no longer significant in the female and male population (Women F=4.909, DF=(2,119), p= 0.01, Men F=1.051, DF=(2,86), p= 0.354). Also, no significant differences among the groups were detected by pairwise comparisons.

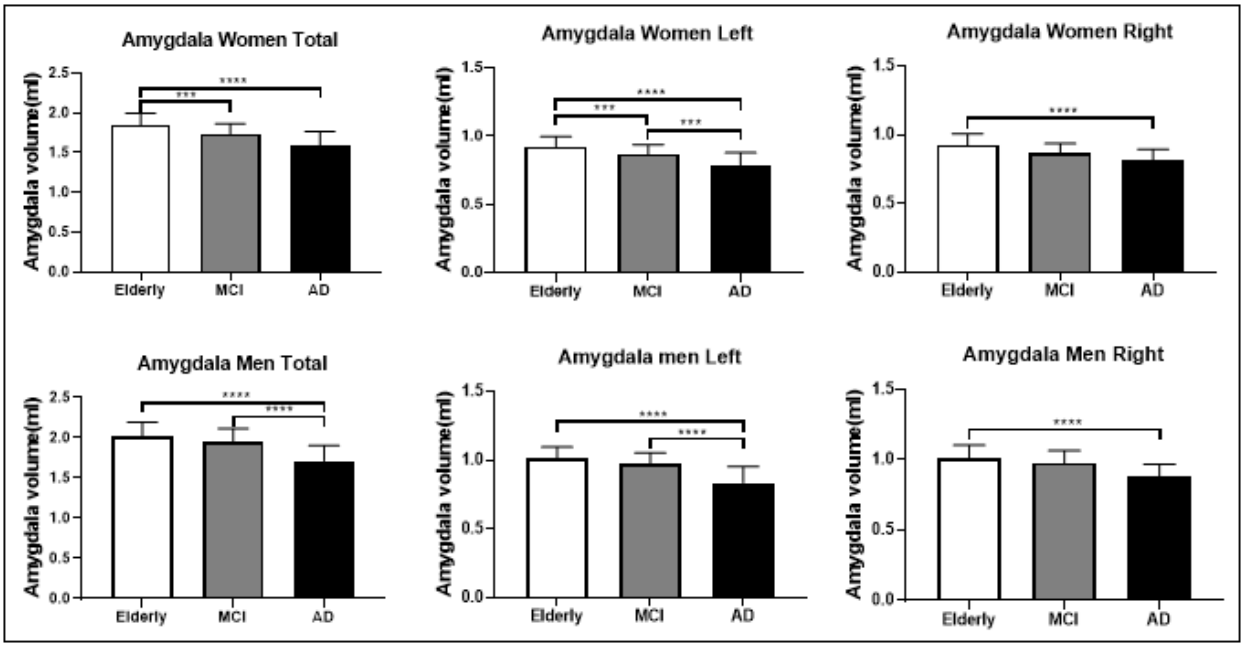


Figure 5.2.4: Amygdala volume differences among the three groups: comparisons among the three groups are shown for the female population (above) and male population (below) separately.

Orbitofrontal Cortex Volume:

The comparison of the volumes of the orbitofrontal cortex among the three groups showed significant differences (F=29.84 and DF=(2,210), p≤0.0001) (figure 5.2.5). *Post-hoc* analyses showed significant differences in the volume of OFC between the elderly and probable AD dementia groups in both sexes in total, left and right volume. The OFC volume in the MCI group was significantly bigger than that of the probable AD dementia group, but only for the male participants in total, left and right volume (Figure 5.2.5, table 5.2.3 for females and table 5.2.4 for males). In the elderly, correlation analyses showed a positive significant correlation between OFC volume and age in men, between OFC

volume and MMSE scores in women and between OFC volume and TIV in both sexes (Table 5.2.5). In the MCI group, a positive association was found between OFC volume and education and between OFC volume and TIV in both sexes and between OFC volume and MMSE scores in women (Table 5.2.6). In the AD group, a positive association was found only between OFC volume and education and between OFC volume and TIV in the female participants (table 5.2.7). ANCOVA controlling for age (Women F=15.824, DF=(2,119), p= 0.0001, Men F=17.562, DF=(2,86), p=0.0001), education (Women F=15.106, DF=(2,119), p=0.0001, Men F=13.043, DF=(2,86), p=0.0001) or TIV (Women F=16.090, DF=(2,119), p=0.0001, Men F=22.418, DF=(2,86), p=0.0001) showed no significant effects of these variables and significant differences among groups persisted. When adjusting for MMSE, groups differences were no longer significant (Women F=3.649, DF=(2,119), p= 0.03, Men F=2.157, DF=(2,86), p= 0.12). No significant differences among groups were detected by pairwise comparisons.

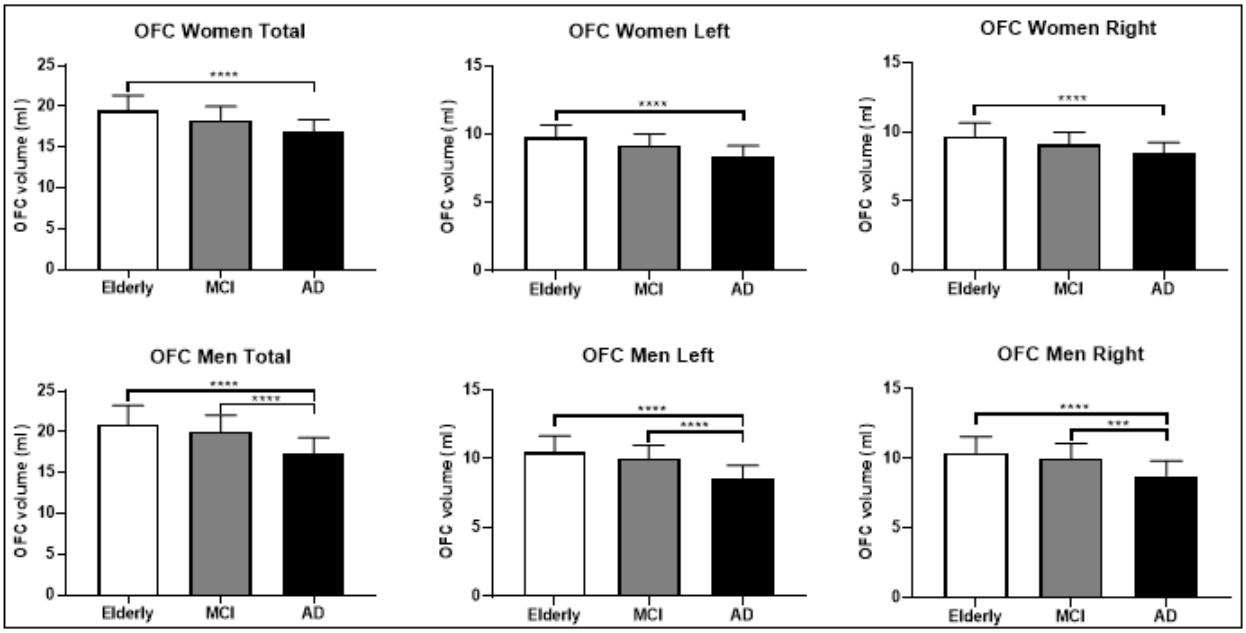


Figure 5.2.5: Orbitofrontal cortex volume difference among the three groups: comparisons among the three groups are shown for the female population (above) and male population (below) separately.

Entorhinal Cortex:

One-way ANOVA analysis showed significant differences in entorhinal volume among the three groups (and F=32.29, DF=(2,210), p≤0.0001) (figure 5.2.6). *Post-hoc* analyses showed a significant difference between the volume of this structure of the probable AD dementia group and that of the elderly controls for both sexes. Furthermore, a significant difference in the volume of this structure was observed between the probable AD dementia patients and the MCI patients in both male and female participants in the left hemisphere. Additionally, the volume of the entorhinal cortex was significantly smaller in the MCI group when compared with that of the elderly controls bilaterally in female participants in the total, left and right volume (Figure 5.2.6, table 5.2.3 for female and

table 5.2.4 for male). Pearson correlation analyses showed a significant negative association in the elderly groups between entorhinal cortex volume and age in men only, a positive correlation between entorhinal cortex volume and MMSE scores in women and a positive correlation between entorhinal cortex volume and TIV in both sexes (table 5.2.5). In the MCI group, entorhinal cortex volume was positively associated with education in men, with MMSE scores in women and with TIV in both male and female patients (table 5.2.6). In contrast, in the probable AD dementia group there was no significant association between entorhinal cortex volume and any variable, except for TIV in women for whom a positive correlation was found (table 5.2.7). ANCOVA including age (Women F=22.495, DF=(2,119), p= 0.0001, Men F=13.359, DF=(2,86), p=0.0001), education (Women F=23.546, DF=(2,119), p=0.0001, Men F=10.685, DF=(2,86), p=0.0001) or TIV (Women F=22.947, DF=(2,119), p=0.0001, Men F=15.373, DF=(2,86), p=0.0001) showed no significant effect of these covariates and significant differences among groups were retained. When adjusting for MMSE, differences in volume of this structure among the three groups were no longer significant in the female and male groups (Women F=5.777, DF=(2,119), p= 0.004, Men F=1.504, DF=(2,86), p= 0.23). Likewise, no significant difference was found in pairwise comparison among the three groups.

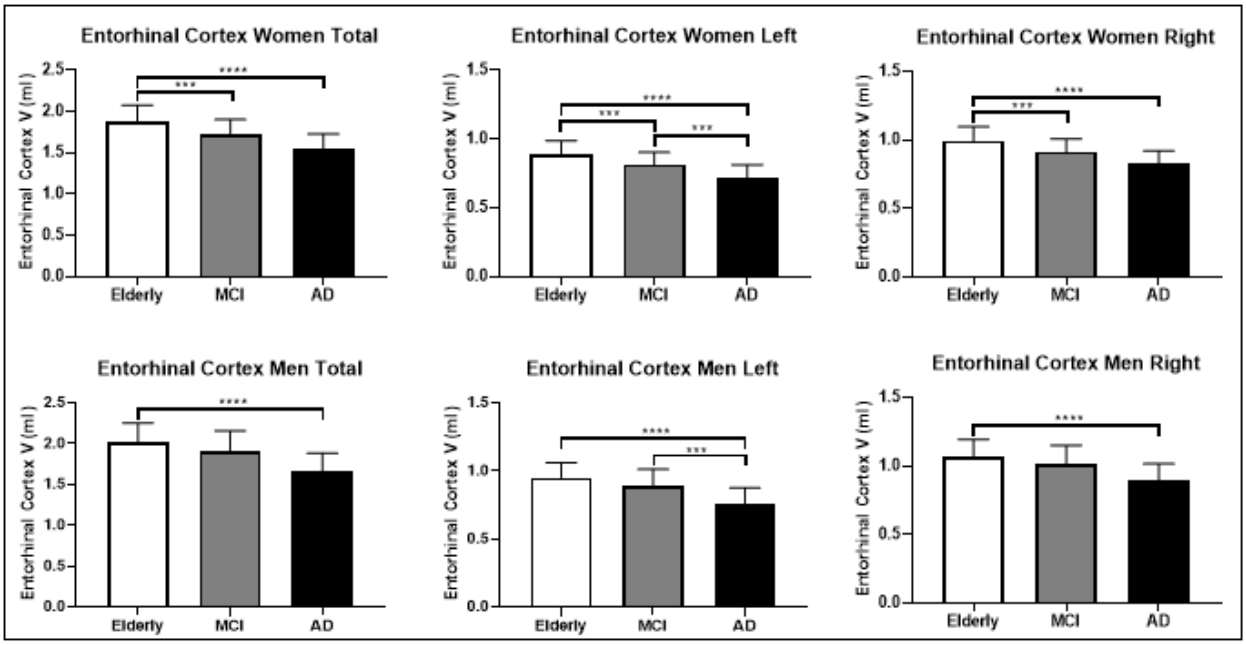


Figure 5.2.6: Entorhinal cortex volume differences among the three groups: comparisons among the three groups are shown for the female population (above) and male population (below) separately.

APOE ε4 carriers vs *APOE* ε4 non-carriers:

A series of t-tests comparing *APOE* ε4 allele carriers and non carriers showed no significant differences in total, left and right OC volume in female and male participants (Table 5.2.8). No significant differences were detected when the groups were split by sex. Similarly, no significant differences were found between the *APOE* ε4 allele carriers and non carriers in hippocampal volume (whether taken as total, split by left and right or when split by sex) (Table 5.2.8). Similar negative findings were shown by the comparisons for the remaining olfactory-related brain regions, i.e. parahippocampus, amygdala, orbitofrontal cortex and entorhinal cortex (whether taken as total, split by left and right or when split by sex) (Table 5.2.8).

Table 5.2.8: Comparisons of olfactory-related brain region volumes between *APOE* ε4 carriers and non-carriers showing no significant differences.

	OCV		Hippocampus		Parahippocampus		Amygdala		OFC		Entorhinal Cortex	
	t	p value	t	p value	t	p value	t	p value	t	p value	t	p value
Women total	**0.26**	**0.79**	**0.43**	**0.67**	**0.18**	**0.86**	**0.49**	**0.62**	**0.47**	**0.64**	**0.04**	**0.97**
Women left	**0.34**	**0.73**	**0.25**	**0.79**	**0.49**	**0.63**	**0.31**	**0.75**	**0.38**	**0.7**	**0.18**	**0.85**
Women right	**0.17**	**0.86**	**0.61**	**0.55**	**0.1**	**0.91**	**0.67**	**0.51**	**0.54**	**0.59**	**0.27**	**0.79**
Men total	**1.18**	**0.24**	**2.26**	**0.03**	**2.5**	**0.02**	**2.28**	**0.03**	**1.85**	**0.07**	**2.63**	**0.01**
Men left	**1.16**	**0.25**	**1.77**	**0.09**	**2.44**	**0.02**	**2.72**	**0.01**	**1.96**	**0.06**	**2.46**	**0.02**
Men Right	**1.19**	**0.24**	**2.45**	**0.02**	**2.47**	**0.2**	**1.87**	**0.07**	**1.73**	**0.09**	**2.66**	**0.01**

APOE ε4 carriers (Women=12, Men=10) non-carriers (Women=19, Men=18) ($p<0.001$). DF (Total=57, women=29 and men=26).

5.2.4 Discussion:

This cohort study investigated whether volumetric differences in olfactory-related brain regions were detectable between healthy elderly controls and patients at different stages of cognitive decline of probable AD aetiology, namely mild cognitive impairment (MCI) stage and mild probable AD dementia. In addition, the effect of carrying the *APOE* ε4 allele on volumetric measures of olfactory-related brain regions was also investigated in a subgroup of participants for whom *APOE* ε4 status was known. Finally, the associations between volumes of olfactory-related brain regions and age, education, MMSE score and TIV was also investigated in all three experimental groups. The use of structural MRI data enabled the study objectives to be met.

The findings of this comprehensive olfactory brain regions study show that olfactory-related brain regions are affected by disease progression in probable AD as well as in MCI. This cohort found a significant volume reduction in the three groups in all brain regions in total, left and right volume in males and females. The female group was more affected as significant volume reductions in some olfactory brain regions such as the hippocampus, parahippocampus, amygdala and entorhinal cortex were already detectable at the level of MCI. Moreover, the left side of the brain appeared to have greater volume reduction than the right hemisphere in AD and MCI. There was an approximately similar pattern of volume reduction between the olfactory cortex and orbitofrontal cortex, and between hippocampus, parahippocampus, amygdala and

partially entorhinal cortex when groups were split by sex. Pathological alterations are observed in the hippocampus, parahippocampus, amygdala and entorhinal cortex, regions that are more involved in odour recognition and identification (Doty et al., 1988; Hawkes, 2003; Iizuka et al., 2021). The reduction observed in this study is in line with previous findings showing olfactory cortex and hippocampus volume reduction in MCI and AD (Vasavada et al., 2015). The present study replicated this finding, and extended them to other olfactory-related brain regions that also showed significant volume reduction. A few studies have shown that olfactory dysfunction is detectable in AD individuals and atrophy of the olfactory bulb and entorhinal cortex is observed early in AD (Devanand et al., 2012; Djordjevic et al., 2008; Thomann, et al., 2009). Moreover, there is some evidence that the olfactory bulb is one of the first brain regions affected by AD pathology (Attems et al., 2014). The finding of this study confirmed that atrophy is found in olfactory related brain regions in probable AD. The reason for deterioration in these olfactory regions is the evidence of early accumulation of neurofibrillary tangles (Attems & Jellinger, 2006). A study demonstrated that pathological changes such as β-Amyloid plaques and neurofibrillary tangles occurred in the olfactory system and its connected regions (Christen-Zaech et al., 2003). Kocahan and Doğan (2017) stated in their review that the pathology occurs in the AD brain and involves change in neurotransmitter expression, reduction of neutrophil numbers, accumulation of senile plaques deposits, neuronal death and atrophy, with this latter being the later stage of the disease (Kocahan & Doğan, 2017). These changes could explain the atrophy shown in this study. Of note, it has been found that the hippocampus is the most affected region in the AD brain (Uysal

& Ozturk, 2020). The olfactory cortex is defined as regions that receive projections from the olfactory bulb. The olfactory bulb is said to be one of the structures first affected in AD. This means that the olfactory cortex should be the most affected in the brain of AD patients. In this study, however, the olfactory cortex appeared less affected than the hippocampus region in our patient groups.

The parahippocampus is connected to the hippocampus as the parahippocampus sends information to the hippocampus via the entorhinal cortex (Naya, 2016) and this might explain the similarity in the atrophy pattern observed in those regions in this study. Another study has recently shown that volume reduction in the entorhinal cortex was associated with parahippocampus volume reduction (Iizuka et al., 2021). There was a similarity of pattern of volumetric reduction between the olfactory cortex and the orbitofrontal cortex. This similarity has been explained by findings showing that the olfactory cortex is connected directly and sends projections to the OFC (Rolls, 2019). Moreover, the medial and posterior OFC has high connectivity with the olfactory cortex and also the OFC posterior border lies next to the olfactory cortex (J. Du et al., 2020). All these might explain the similarity in atrophy pattern shown in those two regions in this study.

Moreover, in olfactory-related brain regions, the left volume was more reduced than the right volume in this study population. This finding supports previous evidence in the literature reporting that the left hemisphere of the brain is more affected by volume reduction and the progression of the disease is more severe on the left side and occurs

earlier in the disease course than in the right hemisphere in the AD brain (Donix et al., 2013; Toga & Thompson, 2003). In fact, a study stated that the brain left side alteration is part of AD development (Gianotti et al., 2007). The same study indicated that MMSE might assess and specifically detect left side functions in the brain (Gianotti et al., 2007). Several studies have also indicated that the left side of the brain is more affected in MCI and AD and that this greater vulnerability to pathology at an early stage of neurodegeneration might be caused by genetic and other disease risk factors (Yu et al., 2019; Zhao et al., 2019). However, it remains still unclear why such a side-based faster deterioration is observed in MCI and/or AD brain.

An important finding is that there was no alteration found between the MCI and control groups in the male population in any of the olfactory-related brain region volumes. Moreover, the reduction in the female MCI group was found only on the left side for all regions of interest except the entorhinal cortex where significant differences were found in both hemispheres. In addition, no reduction in volume was found in the MCI group in OCV or OFC. This means that at the MCI stage no alterations are detectable in olfactory-related brain regions in men while some alterations are detectable in the left hemisphere in female participants. In fact, this finding supports previous evidence that greater atrophy rate is detected in women MCI than men MCI (Nebel et al., 2018) strengthening the findings of this study.

This study shows that age affects the volume of olfactory-related brain regions in men more than in women in the healthy elderly, MCI and probable AD dementia groups.

This correlation was stronger in the elderly, losing gradually its strength in MCI and then in the probable AD group. This finding indicates that ageing is a strong factor in the emergence of an olfactory deficit (Franks et al., 2015). Moreover, in this cohort a strong association was also found between education and the volume of olfactory-related brain regions in the MCI group more than in the healthy elderly control and probable AD dementia groups. An association was also found between MMSE scores and brain volume more in women than in men in the healthy elderly and MCI groups. An association between cognitive abilities and brain volume is a well established finding (Ritchie et al., 2015) that has been established also by the current study. MMSE scores as an index of severity of disease had a strong impact on measures of brain volume of most regions assessed in the current study and when included as a covariate, between group differences were no longer significant. This finding suggests that severity of disease reflected by poor overall cognitive abilities has a strong impact on brain volume loss as expected in this study population. In fact, there is established evidence that change in hippocampus volume, for example, is reflected in the level of cognitive abilities shown by patients and this is also the case when cognitive abilities are assessed using the MMSE in MCI and probable AD dementia patients (Peng et al., 2015).

In the present study, the influence of the genetic risk factor *APOE* ε4 allele was also assessed. The findings show that carrying the *APOE* ε4 allele does not influence any of the olfactory regions volumes selected in this study, as no significant difference in volume was observed between *APOE* ε4 carriers and *APOE* ε4 non-carriers in any of the

olfactory-related brain regions selected. This finding is not in line with previous evidence from studies that showed that *APOE* ε4 carriers show greater brain atrophy than the *APOE* ε4 non-carriers (Reiter et al., 2016). The difference in finding from other studies should, however, be interpreted with caution as the number of participants in the current study for whom a genetic profile for the *APOE* gene was available was very small.

Our study demonstrates that women have greater volumetric loss at an earlier stage of disease than men. Indeed, significant differences in volume from healthy controls were already detectable at the MCI stage in women, while this was not the case for men. This finding supports existing evidence that women experience more severe brain atrophy than men at an earlier stage of disease (Holland et al., 2013). Several factors might be invoked to explain this differential effect of the disease between sexes, with one possible explanation being that women have a more severe biological vulnerability that fosters faster progression of AD (Liesinger et al., 2018; Malpetti et al., 2017). Also, women show a longer duration of the disease (Liesinger et al., 2018). Moreover, women are at higher risk of the disease than men (Nebel et al., 2018) and they are more affected by AD (Mazure & Swendsen, 2016). Furthermore, women are at greater risk of depression with a risk that is double that of men, which increases the risk of developing AD (Nebel et al., 2018). Despite the vast majority of studies being either only done on males, or the data being presented with males and females combined, our study separated the study groups allowing sex related differences to emerge given the premise of differences in physical,

hormonal and abilities between women and men, all of which can have an effect and alter disease trajectories.

Previous studies of olfactory regions have failed to make adjustments in the measurement of brain atrophy for normal ageing effect or for disease severity (Fiford et al., 2018). To the best of our knowledge this is the first study of its kind that has investigated changes in a comprehensive set of neural structures involved in olfaction in healthy elderly and in two diseased groups with a probable common neurodegenerative aetiology. In addition, the present study investigated changes in neural olfactory regions in these groups split by sex and taking a comprehensive approach involving both brain hemispheres using MRI imaging technology.

Because of the wide variability of brain alterations in people in the course of normal ageing, as well as neurodegeneration such that leading to MCI and probable AD dementia, the outcome might be a complex expression of the effects of different variables on atrophy of relevant olfactory regions especially in clinical cases. More research is needed to comprehend the trajectories of atrophy in distinct brain regions fully, both in people ageing normally and in those ageing in a context of disease. The findings of the present study highlight the critical need to investigate the full range of olfactory brain regions to understand in more detail how they are affected by AD pathology. Research should focus on molecular investigations of brain cells in relevant regions to understand better the causes of regional atrophy observed in normally ageing individuals and in those with MCI and probable AD dementia that then lead to olfactory dysfunction.

5.3 Chapter discussion and conclusion:

In this chapter, it became clear that there is reduction in the size of the areas related to the sense of smell in the brain of patients with probable AD and also in patients with mild cognitive impairment. It is also the case that ageing affects the size of these regions of the brain, as shown in chapter 4, but atrophy of these regions appears to be more substantial in people with Alzheimer's disease and cognitive impairment.

It should be noted that the experimental work presented in this chapter shows that there is a similarity in the pattern of atrophy in the parts of the brain associated with the sense of smell, namely the olfactory cortex with orbitofrontal cortex and the hippocampus with parahippocampus, amygdala and partially the entorhinal cortex. This similarity may be due to the spatial connection of these regions, although other unknown factors might also explain this observation.

It is clear in this chapter that in the brain investigated in this study women had more severe volumetric loss at an earlier stage than men, as great atrophy in olfactory related regions was already detected in patients with mild cognitive impairment. Also, in this chapter, it becomes clear to us that the left hemisphere shows greater volume loss than the right side in Alzheimer's patients. This may be from the effect of disease or from the effect of age as well, as the left part is related to many life matters that may negatively affect its volume by virtue of more use and also the fatigue related to that.

Considering the cost, time, and tools needed to carry out proper testing of olfactory functions, it might be more practical and more clinically viable to monitor the volume of

brain regions in the elderly, because this gives indications of Alzheimer's disease and dementia in general, especially the regions related to the sense of smell. If atrophy in these regions is detected in association with early signs of cognitive decline, additional investigations might be offered and if needed preventative strategies put in place to contain the effect of the disease by suggesting a change in diet, regular physical activity and cognitive exercises to help delay or mitigate the effect of this disease.

5.4 Limitation:

The experimental findings reported in this chapter have provided evidence that olfactory-related brain regions are smaller in volume in probable AD when compared with healthy age matched controls. One of the main limitations of this study is its cross-sectional approach. A longitudinal design would have been a more appropriate way to make inferences about progressive tissue loss with increasing severity of disease.

One additional limitation is the relatively small number of participants for whom the *APOE* ε4 allele status was known. The findings of this sub-study, in fact, could have been influenced by lack of statistical power and should be taken with caution.

One final limitation is that the technique that was used allows the volumetric quantification of regional volume but does not allow the identification of the cause and neural breakdowns that determine neuronal loss and volumetric shrinkage. Resolving the neural mechanisms underlying olfactory dysfunction might not be possible in a human cross-sectional design and may require a murine model to discover the cause of volumetric loss in ageing and with progression of a neurodegenerative disease such as AD.

Chapter 6: General discussion and conclusion

The sense of smell is one of the most important senses for living organisms. Through smell, the organism is able to identify its surroundings, in addition to being one of the most important ways for living organisms to interact with external influences: on a pleasant note, someone could enjoy smelling their coffee before drinking it in the morning; on a more hazardous note, someone could smell smoke before seeing a fire or identify spoiled or inedible food before tasting or seeing it. The importance of the sense of smell is highlighted not only in humans but in animals through the detection of the most intense and powerful smells. The nose stimulates internal memory and allows people to remember all the information stored in the brain, and here the importance of the sense of smell emerges, as the sense of smell is closely related to memory. The sense of smell warns of a danger that a person or animal may experience and therefore prompts them to take all necessary precautions to avoid those risks.

In the setting of early AD symptoms and olfactory awareness, experiments in this thesis relied on scarce literature regarding the comprehensive study of olfactory related brain regions in the ageing population, MCI and probable AD dementia patients. Moreover, the use of different ways of testing olfactory capacity and the use of cognitive tests in the same individual as shown in chapter 4, allow this thesis to enrich the literature about olfactory functions of the cognitively healthy elderly population. Importantly, by evaluating olfactory function through objective measures and through olfactory self-

reporting in the same older adult population, as done in some of the experimental work in this thesis, contributes important findings to this area of research.

It is essential to recognise all the deteriorations associated with ageing to protect against the dangers that accompany them, and to make sure that affected individuals have awareness and can implement strategies to compensate for these deficiencies. These deficiencies must be identified and communicated to the elderly, their families, and health care workers. Many of the deteriorations associated with ageing have known causes, but some are still not well recognised.

There is an existing need to study the deterioration in some of the body functions associated with ageing, especially the weakening of the sense of smell, because some people experience differences in cognitive abilities with advancing age and some studies have suggested that these start in early adulthood (Salthouse, 2009). Indeed, there are changes in the shape of the brain with ageing that may cause loss of function (such as cognitive abilities) and neural loss (Fjell & Walhovd, 2010) and thus, knowing and identifying these changes is essential for understanding them as either a part of normal ageing or as the outcome of pathological processes caused by certain diseases such as AD. Moreover, there is an association between olfaction and episodic memory in the elderly (Larsson et al., 2016).

In neurodegenerative disorders, olfactory dysfunction is associated with abnormalities of olfactory brain regions, a finding that has led to olfactory dysfunction being described as a marker of early pathological processes in many neurological

diseases (Christen-Zaech et al., 2003; Doty, 2012; Thomann et al., 2009). Olfactory identification and discrimination are the two olfactory domains that are found to be the earliest affected in the course of AD and may be an indication of onset of cognitive decline (Gilbert et al., 2004; Serby et al., 1991). There are different olfactory domains that might decline in function with ageing and AD; however, identification, discrimination and memory are well studied as being affected in the early stage of AD (Fusetti et al., 2010; Murphy et al., 1999; Serby et al., 1991). Dysfunction in the sense of smell is common in the normal ageing population (Doty & Kamath, 2014). However, it is accelerated in the case of AD.

Alterations in brain volume occur with ageing, and these changes affect functions that are related to the brain, such as the sense of smell. These alterations such as reduction in number and shrinkage in size of brain cells differently lead to a decline in individuals' performance (Fotenos et al., 2008; Raz et al., 2005). Alterations in brain structure begin at a young age and occur primarily in the grey matter (Raz et al., 2005; Terribilli et al., 2011).

One of the main objectives of this thesis was to demonstrate whether self-awareness of olfactory functions is a suitable method of assessment of actual olfactory function in the ageing population by comparing self-report measures with objective olfactory scores obtained on a quantitative olfactory test (see experiment one in chapter 4). When using a quantitative olfactory test (the UPSIT), the results demonstrated that 94% of the participants had olfactory problems. This finding is in line with those of other

studies that have reported that the majority of the elderly individuals tested had olfactory deficits (Murphy, 2002), but the experiment presented in chapter four is one of the first where it has been demonstrated objectively, that self-reporting methods are inadequate to detect such deficits. An alarming finding is that 69% of participants who claimed to have no olfactory dysfunction (95% if we include mild microsomia) were not aware of their actual olfactory dysfunction. This finding suggests that older adults may be exposed to risks in their everyday lives, with no awareness of being at risk. These results also suggest that self-reports as a subjective measure of olfactory ability are neither sensitive nor specific enough to reflect the true olfactory status of an individual.

The results of the clinical study (experiment 1 in chapter 4) suggest that lack of awareness of olfactory dysfunction is very common in the ageing population and the subjective olfaction measurement is not a reliable method for assessing olfactory function in older adults. This finding contradicts the fact that the majority of the literature supports the use of self-reports (Landis et al., 2003; Nordin et al., 1995; Wehling et al., 2011). We conclude that quantitative tests of olfactory function are more reliable and objective when it comes to ascertaining whether a person is experiencing any olfactory dysfunction. An important outcome of this study is that there is no correlation between olfactory self-reports and quantitative tests. Thus, it is important to implement quantitative olfactory testing in clinical settings. It is also important to devise educational programmes for older adults to make them aware of means of non-olfactory detection of hazardous events and to inform them of treatment options for olfactory decline and consequent mental issues that might stem from loss of olfaction (e.g., depression, loss of appetite). Quantitative

olfactory testing would enable reliable levels of olfactory function to be determined in the ageing population to identify whether an individual should go on to have other markers assessed for potential neurodegenerative diseases. A large proportion of older adults are unaware of the deterioration of their sense of smell, and this puts them at risk of hazards in their daily life. Greater effort by families, care homes, and the health system is required to reduce this risk.

Another objective of this thesis was to evaluate the use of two olfactory function tests as quantitative assessment of olfactory function in the ageing population, to test whether there was a link between olfactory dysfunction and cognitive decline (see experiment 2 in chapter 4). Olfactory dysfunction was present in 94% of the ORCA cohort. Deficits in olfactory memory were also present in this cohort. A positive correlation was observed between olfactory function and the scores of both cognitive tests. Moreover, in women who were former smokers, there was a significant association between olfactory short-term memory and cognitive scores. Other studies on olfactory dysfunction have reported similar findings (Murphy, 2002; Wilson, et al., 2007). The sense of smell begins to deteriorate in individuals from the sixth decade of life and older (Doty et al., 1984; Sinding et al., 2014; Wang et al., 2016). This decrease in olfactory function in the elderly population increases the possibility of their encountering and/or being exposed to a possible hazard. The results of the ORCA study showed that deficits in olfactory memory are observed in older adults. The findings are in line with those that have shown that odour memory deficits have also been observed in individuals at risk of AD (Schiffman,

2002) and in healthy elderly carriers of the *APOE* ε4 allele (a risk factor for AD) (Sundermann et al., 2007).

The findings of our study are also supported by previous authors who have reported an association between olfactory function and cognition in older adults (Roberts et al., 2016). Indeed, some studies have suggested that olfactory dysfunction may predict impending cognitive decline (Devanand et al., 2015; Sohrabi et al., 2012; Wilson, Schneider, et al., 2007). Our findings suggest that olfactory dysfunction could predict future cognitive decline, emphasising the importance of treatment in older adults to improve olfactory function.

The final objective of the fourth chapter of this thesis was achieved in experiment 3 that tested whether the olfactory cortex and areas associated with odour processing showed any volumetric loss at differing trajectories as a result of the normal process of ageing, and if there were any sex differences in atrophy or based on *APOE* ε4 carrier status. Our study showed that loss of volume in olfactory related brain regions occurs around the seventh decade in men while in women, this loss was detectable as early as the fourth decade of life, except for volume changes in the hippocampus and amygdala that become noticeable around the seventh decade. This finding confirmed previous evidence that women experience greater brain atrophy than men (Jiang et al., 2014); however this greater volumetric loss appeared to affect all regions in the olfactory neural system except for the hippocampus and amygdala where volumetric loss aligned with that observed in men. Globally, in men, however, volume loss appears to accelerate later

in life, with significant differences seen in comparisons between middle age and elderly groups. This volumetric reduction can be explained by loss of grey matter, the ageing process, stress, or changes in neurotransmitter expression levels (Lindgren et al., 2016; Peters, 2006; Scahill et al., 2003; Shen et al., 2013). Olfactory dysfunction has been correlated with the volumetric reduction of the olfactory cortex, hippocampus, parahippocampus and orbitofrontal cortex in other studies (Bitter et al., 2010). Furthermore, a subsequent study found a significant association between olfactory function and olfactory bulb volume that was observed without age influences (Buschhüter et al., 2008), meaning the smaller the OB volume, the lower the olfactory performance by individuals.

A longitudinal study measuring brain volume using MRI and cognitive abilities in healthy participants with a mean age of 73 years and again at the a mean age of 76 years found that brain tissue volume was associated with cognitive status during normal ageing, suggesting that there is a link between brain volume size and level of cognitive abilities in elderly participants (Ritchie et al., 2015). This study also found that participants who showed higher cognitive ability at the age of 73 years showed less decline in volume during the following three years. In another study, ageing was the highest risk factor for olfactory decline, significantly more than smoking (Doty, 2009; Doty et al., 1984; Zou et al., 2016).

The large and comprehensive study of olfactory brain regions in this thesis covers approximately all adult age groups, taking sex into account and volumetrically measuring

both brain hemispheres using MRI technology. The results of this study suggest that monitoring of the volume of olfactory brain regions in the ageing population may be valuable in detecting early signs of neurodegenerative disease. It is important to measure brain volume among the older adult population as brain volume has consistently been observed to correlate with cognitive ability in older adults (Ritchie et al., 2015).

Another objective of this thesis was to assess whether olfactory-related brain regions, including the olfactory cortex, show volume deterioration in conditions of cognitive decline such as mild cognitive impairment and probable Alzheimer's disease dementia, if there were a different pattern of volume reduction between women and men, and if the presence of the *APOE* ε4 allele affected trajectories of volume reduction (see chapter 5, experiment 4 and the expanded experiment 5). In the pilot study (experiment 4), olfactory cortex volume was lower in both men and women with probable AD dementia as compared with controls. This reduction in volume was observed more in the left olfactory cortex and was influenced by age. An important finding is that there was a significant positive association between level of education and hippocampal volume among the controls and that this association was not detected in the participants with probable AD dementia. Some studies consider education as a proxy of cognitive reserve, exerting its beneficial influence in preserving brain tissue and hence volume as many reports suggest that low education is correlated with increase dementia risk such as AD (Staff et al., 2004). This study by Staff and colleagues considered education and

occupation as proxies of reserve and suggested that those two measures influence different cognitive domains (Staff et al., 2004).

Olfactory dysfunction has been observed in the majority of AD cases (Kashibayashi et al., 2020; Murphy, 2019; Velayudhan, 2015) and is considered an early symptom of the disease (Doty, 2009; Wilson et al., 2009). Moreover, olfactory deficits and atrophy of olfactory related brain regions have been also observed in MCI (Quarmley et al., 2016; Thomann et al., 2009).

As for the greater volumetric reduction in the left hemisphere as compared with the right hemisphere as found in the experiments in this thesis, several studies have demonstrated that left brain regions tend to be more affected in MCI and/or early AD dementia (Barnes et al., 2005; Liang et al., 2018; Loewenstein et al., 1989; Yu et al., 2019; Zhao et al., 2019). No particular reason has been identified however, and several hypotheses have been put forth to explain this left-sided preference, including genetic and disease risk factors such as the presence of the *APOE* ε4 allele. *APOE* ε4 allele(s) carriers may have specific regional differences in their left brain regions (Kelly et al., 2018; Liang et al., 2018; Low et al., 2019), and evidence suggests that the APOE protein may be involved in brain development, physiological and pathological patterns observed later in life (Donix et al., 2013). Moreover, asymmetry (left > right) in atrophy might be caused by accumulation of greater vascular burden in the left hemisphere. It has been found that there are greater levels of vascular burden in the left hemisphere not only in dementia cases but also in non-dementia cases suggesting that this asymmetric neural decline

might be part of normal ageing (Giannakopoulos et al., 2009). The explanation for this is that the left hemisphere might be highly engaged in cognitive task processing that may cause vascular burden as the supply of oxygen and glucose might be insufficient in people with increase vascular burden (Heinzel et al., 2015).

Ageing affects brain size, vasculature, olfaction, and cognition (Peters, 2006). However, higher education level, a healthy diet and exercise have been linked to better cognitive functioning later in life (Peters, 2006). In agreement with the findings of other studies, we noted an association between education level and the volume of the hippocampus in controls (Noble et al., 2012). This association was not detected in the probable AD dementia cases despite no significant difference in education level between the control and AD participants. Lower education levels have been linked to increased exposure to stresses in life, an occurrence that is associated with changes in hippocampal structure (Noble et al., 2012). Since olfactory dysfunction might be one of the early signs of Alzheimer's disease, pathological alterations are observed in the hippocampus, parahippocampus, amygdala and entorhinal cortex regions that are involved in odour recognition and identification (Doty et al., 1988; Hawkes, 2003; Iizuka et al., 2021). Furthermore, the olfactory cortex and the hippocampus show volume reduction in MCI and AD dementia (Vasavada et al., 2015). It is possible that because there are alterations in olfactory related brain regions in AD, olfactory function might be used as an early proxy sign of the presence of Alzheimer’s disease. However, a degree of caution in this respect is needed, since the experiments of this thesis found a strong effect of ageing on those brain regions as well as an effect of disease.

For the olfactory brain regions study (In experiment 5), we demonstrated that olfactory related brain regions are affected by disease progression. This comprehensive olfactory related brain regions study found that these regions are affected to a greater extent by Alzheimer's disease. An approximately similar pattern of volume reduction was found in the olfactory cortex within the orbitofrontal cortex and in the hippocampus with the parahippocampus, amygdala and entorhinal cortex. This volume reduction in olfactory-related brain regions in AD could be the result of the accumulation of neurofibrillary tangles in these regions (Attems & Jellinger, 2006). Moreover, this reduction may be a result of pathological changes such as β-amyloid plaques and neurofibrillary tangles occurring in the olfactory system and its connections (Christen-Zaech et al., 2003). Other explanations have been proposed in the literature to interpret the volume reductions detected in olfactory brain regions in MCI and AD. The fifth experiment in this thesis demonstrated that the deterioration is more pronounced in the left hemisphere than in the right in our study population. This finding has been previously reported, namely that the left side of the brain shows more severe effects of disease than the right side of the brain in early stage Alzheimer's disease (Donix et al., 2013; Toga & Thompson, 2003). As discussed above, several explanations have been offered to interpret this observed asymmetry in the effect of disease on the two brain hemispheres, including a possible effect of carrying the *APOE* ε4 allele, excessive vascular burden, etc.

Our findings also offer support to the presence of an asymmetric volume loss in the olfactory cortex in both the control and disease groups.

No alterations in olfactory regions were found in the MCI group as compared with the control group in the male population. Moreover, while the MCI group showed no alterations in the olfactory brain regions in men, some volume loss in left sided olfactory regions was found in women. These findings support previous reports that there is a greater rate of atrophy in women with MCI than men (Nebel et al., 2018). Our findings showed that women were more affected by illness progression than men, as demonstrated by the presence of a significant difference in volume of the selected structures in women already at the MCI stage when compared with control groups. This research corroborates previous findings that women have more severe brain atrophy than men overall (Holland et al., 2013), and extends these to demonstrate that it occurs at an earlier stage of severity.

Conclusion

This thesis shows that olfaction skills are already disrupted by the normal process of ageing and disruption appears to be exacerbated in the presence of a neurodegenerative conditions such as Alzheimer's disease. This thesis contributes to advancing research in Alzheimer's disease and neurodegenerative conditions in general, specifically strengthening the available methods of early disease detection in susceptible populations, thus contributing to reducing symptoms or even delaying the spread of the disease. Numerous studies have shown that a healthy lifestyle and diet may protect against or reduce the incidence of disease. These studies include a study by Lessard-Beaudoin et al. (2021). This study was meant to be a part of this thesis but, due to circumstances outside the student's control, could not be included. This study indicated that a healthy diet reduced the spread of disease in the body, especially a diet rich in omega-3 fatty acids (Lessard-Beaudoin et al., 2021). Moreover, another relatively recent neuroimaging study has shown that exercise and a healthy lifestyle reduced Alzheimer's disease symptoms (Castellano et al., 2017).

The methodological novelty of this thesis is that it included three olfactory function tests involving subjective and objective tests and two cognitive abilities examinations on the same large number of elderly participants. This allowed the study to identify olfactory dysfunction as well as to pinpoint which domain of olfactory dysfunction was more affected and more strongly associated with cognitive decline in the elderly population. As

shown in the longitudinal study, the objective olfactory test confirmed the inadequacy of subjective reporting in detecting olfactory dysfunction.

Another methodological strength of this thesis is that it is a comprehensive MRI study that includes almost all olfactory-related brain regions measured both across different age categories to detect the effects of normal ageing (the experimental work reported in chapter 4) and of neurodegeneration due to probable Alzheimer's disease (the experimental work reported in chapter 5). To our knowledge there is no study in the literature that has undertaken such a comprehensive volumetric investigation of olfactory related brain regions either in normally ageing individuals or in diseased individuals. The large number of participants in the MRI ageing and disease studies helps us to identify those regions that undergo volume reductions either as a result of physiological brain ageing and/or as a result of neurodegeneration leading to dementia. Future studies, however, should extend these findings beyond volumetric measurements of brain regions of interest and use measures of functional brain activity or brain metabolism to clarify better which neural mechanisms are disrupted either by the physiological process of ageing or by neurodegeneration that, in time, cause olfactory dysfunction to emerge, as well as characterise individual patients' personal circumstances such as head trauma, stressful events, etc that might foster the appearance of this dysfunction.

This thesis has contributed novel findings and clearly demonstrated that subjective reports (self-report) are not useful tools to detect olfactory dysfunction in the older adult population. The findings suggest that objective testing should be used. Moreover, there

is a large portion of the elderly population who has even severe olfactory dysfunction but does not show any awareness of it. This conclusion is based on the evidence coming from combined testing with subjective and objective olfactory measures.

Another important novel outcome is that loss of volume in olfactory related brain regions is affected by age and therefore the study has not been able to demonstrate specificity to AD, although when coupled with some cognitive symptoms, volumetric loss in these regions might be a warning sign of impending AD. These particular regions are sensitive to the natural process of shrinking of the brain; therefore, the neural tissue in these regions naturally declines with age. Overall, the findings suggest that there is a strong effect of ageing, but the evidence from these studies also shows that the effects of the disease are much greater in all of those brain regions related to olfactory function. It would be, therefore, reasonable to suggest that when olfactory dysfunction in an elderly person is coupled with some cognitive deficits it should be investigated further because there is a good chance of presence of probable Alzheimer's disease pathology in the background. This is also advisable at the MCI stage if patients present with severe olfactory dysfunction, since the evidence from this thesis shows that the MCI group had greater volume loss that the control group and the combination of olfactory symptoms and volumetric loss may indicate greater risk of progression to probable AD dementia in this group. Indeed, if signs of this neurodegenerative disease can be detected at a very early stage, appropriate lifestyle changes and management plans could be potentially put in place to fight this condition and limit its spread, resulting potentially in a delay of the

most severe consequences of neurodegeneration and better quality of life for individuals and their families as well as a reduction of cost to health care systems.

One of the advantages of this thesis is that we used the same elderly population data from the ageing study in experiment 3 as a control in the disease study in experiment 5. This means that we are certain that there is olfactory-related brain regions atrophy and yet this control population shows significantly bigger regional volume than the age matched AD group indicating that the disease influence is greater than the ageing influence in the elderly.

Finally, there also is an urgent need to develop new forms of treatment for this disease as there is currently no cure for Alzheimer's disease. However, there are drugs that reduce symptoms of the disease or delay its progress. One of the important aspects of this thesis is that it has contributed some evidence in support of using olfactory dysfunction and volumetric measurements of olfaction-related brain regions as potential indicators of presence of disease that can alert health care providers of potential risk to individuals so that appropriate measures can be implemented to reduce the impact of AD on individuals' lives. This could help to improve the accuracy of AD diagnosis and to identify populations at risk of the disease or to improve the prediction of conversion from MCI to AD.

References:

Abdissa, D., Hamba, N., & Gerbi, A. (2020). Review Article on adult neurogenesis in humans. *Translational Research in Anatomy*, *20*, 100074. https://doi.org/10.1016/j.tria.2020.100074

Abraha, A., Ghoshal, N., Gamblin, T. C., Cryns, V., Berry, R. W., Kuret, J., & Binder, L. I. (2000). C-terminal inhibition of tau assembly in vitro and in Alzheimer's disease. *Journal of Cell Science*, *113 Pt 21*, 3737–3745.

Adams, D. R., Kern, D. W., Wroblewski, K. E., McClintock, M. K., Dale, W., & Pinto, J. M. (2018). Olfactory Dysfunction Predicts Subsequent Dementia in Older U.S. Adults. *Journal of the American Geriatrics Society*, *66*(1), 140–144. https://doi.org/10.1111/jgs.15048

Ajmani, G. S., Suh, H. H., Wroblewski, K. E., & Pinto, J. M. (2017). Smoking and olfactory dysfunction: A systematic literature review and meta-analysis: Review of Smoking and Olfaction. *The Laryngoscope*, *127*(8), 1753–1761. https://doi.org/10.1002/lary.26558

Al Aïn, S., Poupon, D., Hétu, S., Mercier, N., Steffener, J., & Frasnelli, J. (2019). Smell training improves olfactory function and alters brain structure. *NeuroImage*, *189*, 45–54. https://doi.org/10.1016/j.neuroimage.2019.01.008

Alafuzoff, I., Arzberger, T., Al-Sarraj, S., Bodi, I., Bogdanovic, N., Braak, H., Bugiani, O., Del-Tredici, K., Ferrer, I., Gelpi, E., Giaccone, G., Graeber, M. B., Ince, P., Kamphorst, W., King, A., Korkolopoulou, P., Kovács, G. G., Larionov, S., Meyronet, D., … Kretzschmar, H. (2008). Staging of Neurofibrillary Pathology in Alzheimer's Disease: A Study of the BrainNet Europe Consortium. *Brain Pathology*, *0*(0), 080509082911413-??? https://doi.org/10.1111/j.1750-3639.2008.00147.x

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., Gamst, A., Holtzman, D. M., Jagust, W. J., Petersen, R. C., Snyder, P. J., Carrillo, M. C., Thies, B., & Phelps, C. H. (2011).

The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, *7*(3), 270–279. https://doi.org/10.1016/j.jalz.2011.03.008

Al-Otaibi, M., Lessard-Beaudoin, M., Castellano, C.-A., Gris, D., Cunnane, S. C., & Graham, R. K. (2021). Volumetric MRI demonstrates atrophy of the olfactory cortex in AD. *Current Alzheimer Research*, *17*(10), 904–915. https://doi.org/10.2174/1567205017666201215120909

Alves, J., Petrosyan, A., & Magalhães, R. (2014). Olfactory dysfunction in dementia. *World Journal of Clinical Cases*, *2*(11), 661–667. https://doi.org/10.12998/wjcc.v2.i11.661

Alzheimer, A., Stelzmann, R. A., Schnitzlein, H. N., & Murtagh, F. R. (1995). An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde." *Clinical Anatomy (New York, N.Y.)*, *8*(6), 429–431. https://doi.org/10.1002/ca.980080612

Amal, L., Majed, A., Melissa, L.-B., Denis, G., Kate, B., Pierrette, G., & Rona K, G. (2019). Unawareness of Olfactory Dysfunction in Older Adults. *International Journal of Neurology and Neurotherapy*, *6*(2). https://doi.org/10.23937/2378-3001/1410086

Anderson, A. K., Christoff, K., Stappen, I., Panitz, D., Ghahremani, D. G., Glover, G., Gabrieli, J. D. E., & Sobel, N. (2003). Dissociated neural representations of intensity and valence in human olfaction. *Nature Neuroscience*, *6*(2), 196–202. https://doi.org/10.1038/nn1001

Anderton, B. H. (2002). Ageing of the brain. *Mechanisms of Ageing and Development*, *123*(7), 811–817.

Apostolova, L. G., Dinov, I. D., Dutton, R. A., Hayashi, K. M., Toga, A. W., Cummings, J. L., & Thompson, P. M. (2006). 3D comparison of hippocampal atrophy in amnestic mild cognitive impairment and Alzheimer's disease. *Brain*, *129*(11), 2867–2873. https://doi.org/10.1093/brain/awl274

Arnold, S. E., Lee, E. B., Moberg, P. J., Stutzbach, L., Kazi, H., Han, L.-Y., Lee, V. M. Y., & Trojanowski, J. Q. (2010). Olfactory epithelium amyloid-β and paired helical filament-tau pathology in Alzheimer disease. *Annals of Neurology*, *67*(4), 462–469. https://doi.org/10.1002/ana.21910

Attems, J., & Jellinger, K. A. (2006). Olfactory tau pathology in Alzheimer disease and mild cognitive impairment. *Clinical Neuropathology*, *25*(6), 265–271.

Attems, J., Lintner, F., & Jellinger, Kurt. A. (2005). Olfactory involvement in aging and Alzheimer's disease: An autopsy study. *Journal of Alzheimer's Disease*, *7*(2), 149–157. https://doi.org/10.3233/JAD-2005-7208

Attems, J., Walker, L., & Jellinger, K. A. (2014). Olfactory bulb involvement in neurodegenerative diseases. *Acta Neuropathologica*, *127*(4), 459–475. https://doi.org/10.1007/s00401-014-1261-7

Attems, J., Walker, L., & Jellinger, K. A. (2015). Olfaction and Aging: A Mini-Review. *Gerontology*, *61*(6), 485–490. https://doi.org/10.1159/000381619

Babaei, A., Iravani, K., Malekpour, B., Golkhar, B., Soltaniesmaeili, A., & Hosseinialhashemi, M. (2021). Factors associated with anosmia recovery rate in COVID -19 patients. *Laryngoscope Investigative Otolaryngology*, *6*(6), 1248–1255. https://doi.org/10.1002/lio2.690

Bacon, A. W., Bondi, M. W., Salmon, D. P., & Murphy, C. (1998). Very Early Changes in Olfactory Functioning Due to Alzheimer's Disease and the Role of Apolipoprotein E in Olfactiona. *Annals of the New York Academy of Sciences*, *855*(1 OLFACTION AND), 723–731. https://doi.org/10.1111/j.1749-6632.1998.tb10651.x

Bahar-Fuchs, A., Chételat, G., Villemagne, V. L., Moss, S., Pike, K., Masters, C. L., Rowe, C., & Savage, G. (2010). Olfactory deficits and amyloid-β burden in Alzheimer's disease, mild cognitive impairment, and healthy aging: A PiB PET study. *Journal of Alzheimer's Disease: JAD*, *22*(4), 1081–1087. https://doi.org/10.3233/JAD-2010-100696

Bahar-Fuchs, A., Moss, S., Rowe, C., & Savage, G. (2011). Awareness of olfactory deficits in healthy aging, amnestic mild cognitive impairment and Alzheimer's disease. *International Psychogeriatrics*, *23*(07), 1097–1106. https://doi.org/10.1017/S1041610210002371

Baillet, S. (2017). Magnetoencephalography for brain electrophysiology and imaging. *Nature Neuroscience*, *20*(3), 327–339. https://doi.org/10.1038/nn.4504

Baker, E., & Escott-Price, V. (2020). Polygenic Risk Scores in Alzheimer's Disease: Current Applications and Future Directions. *Frontiers in Digital Health*, *2*, 14. https://doi.org/10.3389/fdgth.2020.00014

Barnard, N. D., Bush, A. I., Ceccarelli, A., Cooper, J., de Jager, C. A., Erickson, K. I., Fraser, G., Kesler, S., Levin, S. M., Lucey, B., Morris, M. C., & Squitti, R. (2014). Dietary and lifestyle guidelines for the prevention of Alzheimer's disease. *Neurobiology of Aging*, *35 Suppl 2*, S74-78. https://doi.org/10.1016/j.neurobiolaging.2014.03.033

Barnes, J., Scahill, R. I., Schott, J. M., Frost, C., Rossor, M. N., & Fox, N. C. (2005). Does Alzheimer's Disease Affect Hippocampal Asymmetry? Evidence from a Cross-Sectional and Longitudinal Volumetric MRI Study. *Dementia and Geriatric Cognitive Disorders*, *19*(5–6), 338–344. https://doi.org/10.1159/000084560

Barresi, M., Ciurleo, R., Giacoppo, S., Foti Cuzzola, V., Celi, D., Bramanti, P., & Marino, S. (2012). Evaluation of olfactory dysfunction in neurodegenerative diseases. *Journal of the Neurological Sciences*, *323*(1–2), 16–24. https://doi.org/10.1016/j.jns.2012.08.028

Bath, K. G., Mandairon, N., Jing, D., Rajagopal, R., Kapoor, R., Chen, Z.-Y., Khan, T., Proenca, C. C., Kraemer, R., Cleland, T. A., Hempstead, B. L., Chao, M. V., & Lee, F. S. (2008). Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and

spontaneous olfactory discrimination. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*(10), 2383–2393. https://doi.org/10.1523/JNEUROSCI.4387-07.2008

Belleville, S., Fouquet, C., Duchesne, S., Collins, D. L., Hudon, C., & the CIMA-Q group: Consortium for the Early Identification of Alzheimer's disease-Quebec. (2014). Detecting Early Preclinical Alzheimer's Disease via Cognition, Neuropsychiatry, and Neuroimaging: Qualitative Review and Recommendations for Testing. *Journal of Alzheimer's Disease*, *42*(s4), S375–S382. https://doi.org/10.3233/JAD-141470

Belleville, S., Fouquet, C., Hudon, C., Zomahoun, H. T. V., Croteau, J., & Consortium for the Early Identification of Alzheimer's disease-Quebec. (2017). Neuropsychological Measures that Predict Progression from Mild Cognitive Impairment to Alzheimer's type dementia in Older Adults: A Systematic Review and Meta-Analysis. *Neuropsychology Review*, *27*(4), 328–353. https://doi.org/10.1007/s11065-017-9361-5

Bernhardson, B.-M., Tishelman, C., & Rutqvist, L. E. (2008). Self-reported taste and smell changes during cancer chemotherapy. *Supportive Care in Cancer*, *16*(3), 275–283. https://doi.org/10.1007/s00520-007-0319-7

Beshir, S. A., Aadithsoorya, A. M., Parveen, A., Goh, S. S. L., Hussain, N., & Menon, V. B. (2022). Aducanumab Therapy to Treat Alzheimer's Disease: A Narrative Review. *International Journal of Alzheimer's Disease*, *2022*, 9343514. https://doi.org/10.1155/2022/9343514

Bessac, B. F., & Jordt, S.-E. (2010). Sensory detection and responses to toxic gases: Mechanisms, health effects, and countermeasures. *Proceedings of the American Thoracic Society*, *7*(4), 269–277. https://doi.org/10.1513/pats.201001-004SM

Besser, G., Jobs, L., Liu, D. T., Mueller, C. A., & Renner, B. (2019). The Sniffin' Sticks Odor Discrimination Memory Test: A Rapid, Easy-to-Use, Reusable Procedure for Testing Olfactory Memory. *Annals*

of Otology, Rhinology & Laryngology, *128*(3), 227–232. https://doi.org/10.1177/0003489418818189

Bitter, T., Gudziol, H., Burmeister, H. P., Mentzel, H. J., Guntinas-Lichius, O., & Gaser, C. (2010). Anosmia Leads to a Loss of Gray Matter in Cortical Brain Areas. *Chemical Senses*, *35*(5), 407–415. https://doi.org/10.1093/chemse/bjq028

Boesveldt, S., Postma, E. M., Boak, D., Welge-Luessen, A., Schöpf, V., Mainland, J. D., Martens, J., Ngai, J., & Duffy, V. B. (2017). Anosmia-A Clinical Review. *Chemical Senses*, *42*(7), 513–523. https://doi.org/10.1093/chemse/bjx025

Boughey, J. G. F., & Graff-Radford, N. R. (1987). ALZHEIMER'S DISEASE. In *Neurology and Clinical Neuroscience* (pp. 846–858). Elsevier. https://doi.org/10.1016/B978-0-323-03354-1.50069-9

Boyle, P. A., Wilson, R. S., Aggarwal, N. T., Tang, Y., & Bennett, D. A. (2006). Mild cognitive impairment: Risk of Alzheimer disease and rate of cognitive decline. *Neurology*, *67*(3), 441–445. https://doi.org/10.1212/01.wnl.0000228244.10416.20

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, *82*(4), 239–259.

Braak, H., Braak, E., Yilmazer, D., de Vos, R. A. I., Jansen, E. N. H., & Bohl, J. (1996). Pattern of brain destruction in Parkinson's and Alzheimer's diseases. *Journal of Neural Transmission*, *103*(4), 455–490. https://doi.org/10.1007/BF01276421

Brämerson, A., Johansson, L., Ek, L., Nordin, S., & Bende, M. (2004). Prevalence of Olfactory Dysfunction: The skövde Population-Based Study: *The Laryngoscope*, *114*(4), 733–737. https://doi.org/10.1097/00005537-200404000-00026

Brattoli, M., de Gennaro, G., de Pinto, V., Loiotile, A. D., Lovascio, S., & Penza, M. (2011). Odour detection methods: Olfactometry and chemical sensors. *Sensors (Basel, Switzerland)*, *11*(5), 5290–5322. https://doi.org/10.3390/s110505290

Brier, M. R., Gordon, B., Friedrichsen, K., McCarthy, J., Stern, A., Christensen, J., Owen, C., Aldea, P., Su, Y., Hassenstab, J., Cairns, N. J., Holtzman, D. M., Fagan, A. M., Morris, J. C., Benzinger, T. L. S., & Ances, B. M. (2016). Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. *Science Translational Medicine*, *8*(338), 338ra66-338ra66. https://doi.org/10.1126/scitranslmed.aaf2362

Buchman, A. S., Yu, L., Boyle, P. A., Schneider, J. A., De Jager, P. L., & Bennett, D. A. (2016). Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. *Neurology*, *86*(8), 735–741. https://doi.org/10.1212/WNL.0000000000002387

Burke, S. L., Hu, T., Fava, N. M., Li, T., Rodriguez, M. J., Schuldiner, K. L., Burgess, A., & Laird, A. (2019). Sex differences in the development of mild cognitive impairment and probable Alzheimer's disease as predicted by hippocampal volume or white matter hyperintensities. *Journal of Women & Aging*, *31*(2), 140–164. https://doi.org/10.1080/08952841.2018.1419476

Buschhüter, D., Smitka, M., Puschmann, S., Gerber, J. C., Witt, M., Abolmaali, N. D., & Hummel, T. (2008). Correlation between olfactory bulb volume and olfactory function. *NeuroImage*, *42*(2), 498–502. https://doi.org/10.1016/j.neuroimage.2008.05.004

Butterfield, D. A., Swomley, A. M., & Sultana, R. (2013). Amyloid *β* -Peptide (1–42)-Induced Oxidative Stress in Alzheimer Disease: Importance in Disease Pathogenesis and Progression. *Antioxidants & Redox Signaling*, *19*(8), 823–835. https://doi.org/10.1089/ars.2012.5027

Cain, W. S., Stevens, J. C., Nickou, C. M., Giles, A., Johnston, I., & Garcia-Medina, M. R. (1995). Life-span development of odor identification, learning, and olfactory sensitivity. *Perception*, *24*(12), 1457–1472. https://doi.org/10.1068/p241457

Calhoun-Haney, R., & Murphy, C. (2005). Apolipoprotein ε4 is associated with more rapid decline in odor identification than in odor threshold or Dementia Rating Scale scores. *Brain and Cognition*, *58*(2), 178–182. https://doi.org/10.1016/j.bandc.2004.10.004

Canada. (2012). *Living arrangements of seniors*. Minister of Industry. http://www12.statcan.gc.ca/census-recensement/2011/as-sa/98-312-x/98-312-x2011003_4-eng.cfm

Cárdenas, A. M., Ardiles, A. O., Barraza, N., Baéz-Matus, X., & Caviedes, P. (2012). Role of Tau Protein in Neuronal Damage in Alzheimer's Disease and Down Syndrome. *Archives of Medical Research*, *43*(8), 645–654. https://doi.org/10.1016/j.arcmed.2012.10.012

Carmichael, S. T., Clugnet, M.-C., & Price, J. L. (1994). Central olfactory connections in the macaque monkey. *The Journal of Comparative Neurology*, *346*(3), 403–434. https://doi.org/10.1002/cne.903460306

Carvalho, D. Z., St Louis, E. K., Knopman, D. S., Boeve, B. F., Lowe, V. J., Roberts, R. O., Mielke, M. M., Przybelski, S. A., Machulda, M. M., Petersen, R. C., Jack, C. R., & Vemuri, P. (2018). Association of Excessive Daytime Sleepiness With Longitudinal β-Amyloid Accumulation in Elderly Persons Without Dementia. *JAMA Neurology*, *75*(6), 672. https://doi.org/10.1001/jamaneurol.2018.0049

Cassano, T., Romano, A., Macheda, T., Colangeli, R., Cimmino, C. S., Petrella, A., LaFerla, F. M., Cuomo, V., & Gaetani, S. (2011). Olfactory memory is impaired in a triple transgenic model of Alzheimer disease. *Behavioural Brain Research*, *224*(2), 408–412. https://doi.org/10.1016/j.bbr.2011.06.029

Castellano, C.-A., Paquet, N., Dionne, I. J., Imbeault, H., Langlois, F., Croteau, E., Tremblay, S., Fortier, M., Matte, J. J., Lacombe, G., Fülöp, T., Bocti, C., & Cunnane, S. C. (2017). A 3-Month Aerobic Training Program Improves Brain Energy Metabolism in Mild Alzheimer's Disease: Preliminary Results from a Neuroimaging Study. *Journal of Alzheimer's Disease*, *56*(4), 1459–1468. https://doi.org/10.3233/JAD-161163

Cermakova, P., Eriksdotter, M., Lund, L. H., Winblad, B., Religa, P., & Religa, D. (2015). Heart failure and Alzheimer's disease. *Journal of Internal Medicine*, *277*(4), 406–425. https://doi.org/10.1111/joim.12287

Chabrier, M. A., Blurton-Jones, M., Agazaryan, A. A., Nerhus, J. L., Martinez-Coria, H., & LaFerla, F. M. (2012). Soluble A Promotes Wild-Type Tau Pathology In Vivo. *Journal of Neuroscience*, *32*(48), 17345–17350. https://doi.org/10.1523/JNEUROSCI.0172-12.2012

Chaudhury, S., Brookes, K. J., Patel, T., Fallows, A., Guetta-Baranes, T., Turton, J. C., Guerreiro, R., Bras, J., Hardy, J., Francis, P. T., Croucher, R., Holmes, C., Morgan, K., & Thomas, A. J. (2019). Alzheimer's disease polygenic risk score as a predictor of conversion from mild-cognitive impairment. *Translational Psychiatry*, *9*(1), 154. https://doi.org/10.1038/s41398-019-0485-7

Chetelat, G. aël, & Baron, J.-C. (2003). Early diagnosis of alzheimer's disease: Contribution of structural neuroimaging. *NeuroImage*, *18*(2), 525–541. https://doi.org/10.1016/S1053-8119(02)00026-5

Choi, S. H., & Tanzi, R. E. (2019). Is Alzheimer's Disease a Neurogenesis Disorder? *Cell Stem Cell*, *25*(1), 7–8. https://doi.org/10.1016/j.stem.2019.06.001

Choudhury, E. S. (2003). Influences of Age and Sex on a Microencapsulated Odor Memory Test. *Chemical Senses*, *28*(9), 799–805. https://doi.org/10.1093/chemse/bjg072

Christen-Zaech, S., Kraftsik, R., Pillevuit, O., Kiraly, M., Martins, R., Khalili, K., & Miklossy, J. (2003). Early Olfactory Involvement in Alzheimer's Disease. *Canadian Journal of Neurological Sciences /*

Journal Canadien Des Sciences Neurologiques, *30*(01), 20–25. https://doi.org/10.1017/S0317167100002389

Chu, J., Lauretti, E., & Praticò, D. (2017). Caspase-3-dependent cleavage of Akt modulates tau phosphorylation via GSK3β kinase: Implications for Alzheimer's disease. *Molecular Psychiatry*, *22*(7), 1002–1008. https://doi.org/10.1038/mp.2016.214

Chung, M. S., Choi, W. R., Jeong, H.-Y., Lee, J. H., & Kim, J. H. (2018). MR Imaging–Based Evaluations of Olfactory Bulb Atrophy in Patients with Olfactory Dysfunction. *American Journal of Neuroradiology*, *39*(3), 532–537. https://doi.org/10.3174/ajnr.A5491

Ciofalo, A., Filiaci, F., Romeo, R., Zambetti, G., & Vestri, A. R. (2006). Epidemiological aspects of olfactory dysfunction. *Rhinology*, *44*(1), 78–82.

Citron, B. A., Dennis, J. S., Zeitlin, R. S., & Echeverria, V. (2008). Transcription factor Sp1 dysregulation in Alzheimer's disease. *Journal of Neuroscience Research*, *86*(11), 2499–2504. https://doi.org/10.1002/jnr.21695

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D. X., Hu, L., & Kramer, A. F. (2006). Aerobic Exercise Training Increases Brain Volume in Aging Humans. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *61*(11), 1166–1170. https://doi.org/10.1093/gerona/61.11.1166

Conen, D., Everett, B. M., Kurth, T., Creager, M. A., Buring, J. E., Ridker, P. M., & Pradhan, A. D. (2011). Smoking, Smoking Cessation, and Risk for Symptomatic Peripheral Artery Disease in Women: A Cohort Study. *Annals of Internal Medicine*, *154*(11), 719. https://doi.org/10.7326/0003-4819-154-11-201106070-00003

Cook, A., Pfeiffer, L.-M., Thiele, S., Coenen, V. A., & Döbrössy, M. D. (2017). Olfactory discrimination and memory deficits in the Flinders Sensitive Line rodent model of depression. *Behavioural Processes*, *143*, 25–29. https://doi.org/10.1016/j.beproc.2017.08.006

Courchesne, E., Chisum, H. J., Townsend, J., Cowles, A., Covington, J., Egaas, B., Harwood, M., Hinds, S., & Press, G. A. (2000). Normal Brain Development and Aging: Quantitative Analysis at in Vivo MR Imaging in Healthy Volunteers. *Radiology*, *216*(3), 672–682. https://doi.org/10.1148/radiology.216.3.r00au37672

Crivello, F., Lemaître, H., Dufouil, C., Grassiot, B., Delcroix, N., Tzourio-Mazoyer, N., Tzourio, C., & Mazoyer, B. (2010). Effects of ApoE-ε4 allele load and age on the rates of grey matter and hippocampal volumes loss in a longitudinal cohort of 1186 healthy elderly persons. *NeuroImage*, *53*(3), 1064–1069. https://doi.org/10.1016/j.neuroimage.2009.12.116

Crivello, F., Tzourio-Mazoyer, N., Tzourio, C., & Mazoyer, B. (2014). Longitudinal Assessment of Global and Regional Rate of Grey Matter Atrophy in 1,172 Healthy Older Adults: Modulation by Sex and Age. *PLoS ONE*, *9*(12), e114478. https://doi.org/10.1371/journal.pone.0114478

Croteau, E., Castellano, C. A., Fortier, M., Bocti, C., Fulop, T., Paquet, N., & Cunnane, S. C. (2017). A cross-sectional comparison of brain glucose and ketone metabolism in cognitively healthy older adults, mild cognitive impairment and early Alzheimer's disease. *Experimental Gerontology*. https://doi.org/10.1016/j.exger.2017.07.004

Croy, I., Zehner, C., Larsson, M., Zucco, G. M., & Hummel, T. (2015). Test-Retest Reliability and Validity of the Sniffin' TOM Odor Memory Test. *Chemical Senses*, *40*(3), 173–179. https://doi.org/10.1093/chemse/bju069

da Silva, G. F. Z., & Ming, L.-J. (2007). Metallo-ROS in Alzheimer's Disease: Oxidation of Neurotransmitters by CuII-β-Amyloid and Neuropathology of the Disease. *Angewandte Chemie International Edition*, *46*(18), 3337–3341. https://doi.org/10.1002/anie.200604421

de la Monte, S. M., & Wands, J. R. (2004). Alzheimer-associated neuronal thread protein mediated cell death is linked to impaired insulin signaling. *Journal of Alzheimer's Disease: JAD*, *6*(3), 231–242.

De Marco, M., Beltrachini, L., Biancardi, A., Frangi, A. F., & Venneri, A. (2017). Machine-learning Support to Individual Diagnosis of Mild Cognitive Impairment Using Multimodal MRI and Cognitive Assessments. *Alzheimer Disease & Associated Disorders*, *31*(4), 278–286. https://doi.org/10.1097/WAD.0000000000000208

De Marco, M., Ourselin, S., & Venneri, A. (2019). Age and hippocampal volume predict distinct parts of default mode network activity. *Scientific Reports*, *9*(1), 16075. https://doi.org/10.1038/s41598-019-52488-9

De Vos, A., Struyfs, H., Jacobs, D., Fransen, E., Klewansky, T., De Roeck, E., Robberecht, C., Van Broeckhoven, C., Duyckaerts, C., Engelborghs, S., & Vanmechelen, E. (2016). The Cerebrospinal Fluid Neurogranin/BACE1 Ratio is a Potential Correlate of Cognitive Decline in Alzheimer's Disease. *Journal of Alzheimer's Disease*, *53*(4), 1523–1538. https://doi.org/10.3233/JAD-160227

DeCarli, C. (2007). Qualitative Estimates of Medial Temporal Atrophy as a Predictor of Progression From Mild Cognitive Impairment to Dementia. *Archives of Neurology*, *64*(1), 108. https://doi.org/10.1001/archneur.64.1.108

Dementia. (2017). http://www.who.int/news-room/fact-sheets/detail/dementia

Dennis, E. L., & Thompson, P. M. (2014). Functional Brain Connectivity Using fMRI in Aging and Alzheimer's Disease. *Neuropsychology Review*, *24*(1), 49–62. https://doi.org/10.1007/s11065-014-9249-6

Desiato, V. M., Levy, D. A., Byun, Y. J., Nguyen, S. A., Soler, Z. M., & Schlosser, R. J. (2021). The Prevalence of Olfactory Dysfunction in the General Population: A Systematic Review and Meta-analysis. *American Journal of Rhinology & Allergy*, *35*(2), 195–205. https://doi.org/10.1177/1945892420946254

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*, *31*(3), 968–980. https://doi.org/10.1016/j.neuroimage.2006.01.021

Devanand, D. P., Bansal, R., Liu, J., Hao, X., Pradhaban, G., & Peterson, B. S. (2012). MRI hippocampal and entorhinal cortex mapping in predicting conversion to Alzheimer's disease. *NeuroImage*, *60*(3), 1622–1629. https://doi.org/10.1016/j.neuroimage.2012.01.075

Devanand, D. P., Lee, S., Manly, J., Andrews, H., Schupf, N., Doty, R. L., Stern, Y., Zahodne, L. B., Louis, E. D., & Mayeux, R. (2015). Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology*, *84*(2), 182–189. https://doi.org/10.1212/WNL.0000000000001132

Devanand, D. P., Liu, X., Chunga, R. E., Cohen, H., Andrews, H., Schofield, P. W., Stern, Y., Huey, E. D., Choi, J., & Pelton, G. H. (2020). Odor Identification Impairment and Change with Cholinesterase Inhibitor Treatment in Mild Cognitive Impairment. *Journal of Alzheimer's Disease*, *75*(3), 845–854. https://doi.org/10.3233/JAD-200021

Devanand, D. P., Michaels-Marston, K. S., Liu, X., Pelton, G. H., Padilla, M., Marder, K., Bell, K., Stern, Y., & Mayeux, R. (2000). Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. *The American Journal of Psychiatry*, *157*(9), 1399–1405. https://doi.org/10.1176/appi.ajp.157.9.1399

Devanand, D. P., Pradhaban, G., Liu, X., Khandji, A., De Santi, S., Segal, S., Rusinek, H., Pelton, G. H., Honig, L. S., Mayeux, R., Stern, Y., Tabert, M. H., & de Leon, M. J. (2007). Hippocampal and entorhinal atrophy in mild cognitive impairment: Prediction of Alzheimer disease. *Neurology*, *68*(11), 828–836. https://doi.org/10.1212/01.wnl.0000256697.20968.d7

Dias, H. K. I., Brown, C. L. R., Polidori, M. C., Lip, G. Y. H., & Griffiths, H. R. (2015). LDL-lipids from patients with hypercholesterolaemia and Alzheimer's disease are inflammatory to microvascular endothelial cells: Mitigation by statin intervention. *Clinical Science*, *129*(12), 1195–1206. https://doi.org/10.1042/CS20150351

Dinc, A. S., Sengezer, T., Cayonu, M., & Sahin, M. M. (2020). Smoking cessation improves olfactory functions. *The Laryngoscope*, *130*(2). https://doi.org/10.1002/lary.27992

Djordjevic, J., Jones-Gotman, M., De Sousa, K., & Chertkow, H. (2008). Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of Aging*, *29*(5), 693–706. https://doi.org/10.1016/j.neurobiolaging.2006.11.014

Doan, N. T., Engvig, A., Zaske, K., Persson, K., Lund, M. J., Kaufmann, T., Cordova-Palomera, A., Alnæs, D., Moberget, T., Brækhus, A., Barca, M. L., Nordvik, J. E., Engedal, K., Agartz, I., Selbæk, G., Andreassen, O. A., & Westlye, L. T. (2017). Distinguishing early and late brain aging from the Alzheimer's disease spectrum: Consistent morphological patterns across independent samples. *NeuroImage*, *158*, 282–295. https://doi.org/10.1016/j.neuroimage.2017.06.070

Donix, M., Burggren, A. C., Scharf, M., Marschner, K., Suthana, N. A., Siddarth, P., Krupa, A. K., Jones, M., Martin-Harris, L., Ercoli, L. M., Miller, K. J., Werner, A., von Kummer, R., Sauer, C., Small, G. W., Holthoff, V. A., & Bookheimer, S. Y. (2013). APOE associated hemispheric asymmetry of entorhinal cortical thickness in aging and Alzheimer's disease. *Psychiatry Research: Neuroimaging*, *214*(3), 212–220. https://doi.org/10.1016/j.pscychresns.2013.09.006

Donovan, N. J., Locascio, J. J., Marshall, G. A., Gatchel, J., Hanseeuw, B. J., Rentz, D. M., Johnson, K. A., Sperling, R. A., & for the Harvard Aging Brain Study. (2018). Longitudinal Association of Amyloid Beta and Anxious-Depressive Symptoms in Cognitively Normal Older Adults. *American Journal of Psychiatry*, *175*(6), 530–537. https://doi.org/10.1176/appi.ajp.2017.17040442

Doty, R. (2009). The Olfactory System and Its Disorders. *Seminars in Neurology*, *29*(01), 074–081. https://doi.org/10.1055/s-0028-1124025

Doty, R. L. (2003). *The Odor Memory Test administration manual* (2nd ed.). Sensonics.

Doty, R. L. (2012). Olfaction in Parkinson's disease and related disorders. *Neurobiology of Disease*, *46*(3), 527–552. https://doi.org/10.1016/j.nbd.2011.10.026

Doty, R. L. (2017a). Sense of Smell. In *Reference Module in Neuroscience and Biobehavioral Psychology* (p. B9780128093245065000). Elsevier. https://doi.org/10.1016/B978-0-12-809324-5.06559-7

Doty, R. L. (2017b). Olfactory dysfunction in neurodegenerative diseases: Is there a common pathological substrate? *The Lancet Neurology*, *16*(6), 478–488. https://doi.org/10.1016/S1474-4422(17)30123-0

Doty, R. L., Deems, D. A., & Stellar, S. (1988). Olfactory dysfunction in parkinsonism: A general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology*, *38*(8), 1237–1237. https://doi.org/10.1212/WNL.38.8.1237

Doty, R. L., Frye, R. E., & Agrawal, U. (1989). Internal consistency reliability of the fractionated and whole University of Pennsylvania Smell Identification Test. *Perception & Psychophysics*, *45*(5), 381–384. https://doi.org/10.3758/BF03210709

Doty, R. L., & Kamath, V. (2014). The influences of age on olfaction: A review. *Frontiers in Psychology*, *5*. https://doi.org/10.3389/fpsyg.2014.00020

Doty, R. L., Marcus, A., & William Lee, W. (1996). Development of the 12-Item Cross-Cultural Smell Identification Test(CC-SIT). *The Laryngoscope*, *106*(3), 353–356. https://doi.org/10.1097/00005537-199603000-00021

Doty, R. L., Perl, D. P., Steele, J. C., Chen, K. M., Pierce, J. D., Reyes, P., & Kurland, L. T. (1991). Olfactory dysfunction in three neurodegenerative diseases. *Geriatrics*, *46 Suppl 1*, 47–51.

Doty, R. L., Reyes, P. F., & Gregor, T. (1987). Presence of both odor identification and detection deficits in Alzheimer's disease. *Brain Research Bulletin*, *18*(5), 597–600. https://doi.org/10.1016/0361-9230(87)90129-8

Doty, R. L., Shaman, P., Applebaum, S. L., Giberson, R., Siksorski, L., & Rosenberg, L. (1984). Smell identification ability: Changes with age. *Science (New York, N.Y.)*, *226*(4681), 1441–1443. https://doi.org/10.1126/science.6505700

Doty, R. L., Shaman, P., & Dann, M. (1984). Development of the university of pennsylvania smell identification test: A standardized microencapsulated test of olfactory function. *Physiology & Behavior*, *32*(3), 489–502. https://doi.org/10.1016/0031-9384(84)90269-5

Doty, R. L., Tourbier, I., Ng, V., Neff, J., Armstrong, D., Battistini, M., Sammel, M. D., Gettes, D., Evans, D. L., Mirza, N., Moberg, P. J., Connolly, T., & Sondheimer, S. J. (2015). Influences of hormone replacement therapy on olfactory and cognitive function in postmenopausal women. *Neurobiology of Aging*, *36*(6), 2053–2059. https://doi.org/10.1016/j.neurobiolaging.2015.02.028

Doty, R., Shaman, P., Applebaum, S., Giberson, R., Siksorski, L., & Rosenberg, L. (1984). Smell identification ability: Changes with age. *Science*, *226*(4681), 1441–1443. https://doi.org/10.1126/science.6505700

Du, A. T., Schuff, N., Kramer, J. H., Ganzer, S., Zhu, X. P., Jagust, W. J., Miller, B. L., Reed, B. R., Mungas, D., Yaffe, K., Chui, H. C., & Weiner, M. W. (2004). Higher atrophy rate of entorhinal cortex than hippocampus in AD. *Neurology*, *62*(3), 422–427.

Du, J., Rolls, E. T., Cheng, W., Li, Y., Gong, W., Qiu, J., & Feng, J. (2020). Functional connectivity of the orbitofrontal cortex, anterior cingulate cortex, and inferior frontal gyrus in humans. *Cortex*, *123*, 185–199. https://doi.org/10.1016/j.cortex.2019.10.012

Dubois, B., Feldman, H. H., Jacova, C., DeKosky, S. T., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P. J., & Scheltens, P. (2007). Research criteria for the diagnosis of Alzheimer's disease: Revising the NINCDS–ADRDA criteria. *The Lancet Neurology*, *6*(8), 734–746. https://doi.org/10.1016/S1474-4422(07)70178-3

Dubois, B., Feldman, H. H., Jacova, C., Hampel, H., Molinuevo, J. L., Blennow, K., DeKosky, S. T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G. B., Fox, N. C., Galasko, D., Habert, M.-O., Jicha, G. A., Nordberg, A., … Cummings, J. L. (2014). Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *The Lancet Neurology*, *13*(6), 614–629. https://doi.org/10.1016/S1474-4422(14)70090-0

Duff, K., McCaffrey, R. J., & Solomon, G. S. (2002). The Pocket Smell Test: Successfully discriminating probable Alzheimer's dementia from vascular dementia and major depression. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *14*(2), 197–201. https://doi.org/10.1176/jnp.14.2.197

Duff, K., Tometich, D., & Dennett, K. (2015). The Modified Telephone Interview for Cognitive Status is More Predictive of Memory Abilities Than the Mini-Mental State Examination. *Journal of*

Geriatric Psychiatry and Neurology, *28*(3), 193–197. https://doi.org/10.1177/0891988715573532

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function. *Cell*, *112*(2), 257–269. https://doi.org/10.1016/S0092-8674(03)00035-7

Embong, M. F., Yaacob, R., Abdullah, M. S., Abdul Karim, A. H., Ghazali, A. K., & Jalaluddin, W. M. S. (2013). MR Volumetry of Hippocampus in Normal Adult Malay of Age 50 Years Old and Above. *The Malaysian Journal of Medical Sciences: MJMS*, *20*(4), 25–31.

Engemann, D. A., Kozynets, O., Sabbagh, D., Lemaître, G., Varoquaux, G., Liem, F., & Gramfort, A. (2020). Combining magnetoencephalography with magnetic resonance imaging enhances learning of surrogate-biomarkers. *ELife*, *9*, e54055. https://doi.org/10.7554/eLife.54055

Eriksen, J. L., Sagi, S. A., Smith, T. E., Weggen, S., Das, P., McLendon, D. C., Ozols, V. V., Jessing, K. W., Zavitz, K. H., Koo, E. H., & Golde, T. E. (2003). NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. *The Journal of Clinical Investigation*, *112*(3), 440–449. https://doi.org/10.1172/JCI18162

Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.-M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, *4*(11), 1313–1317. https://doi.org/10.1038/3305

Escott-Price, V., Myers, A. J., Huentelman, M., & Hardy, J. (2017). Polygenic risk score analysis of pathologically confirmed Alzheimer disease: PRS Analysis of AD. *Annals of Neurology*, *82*(2), 311–314. https://doi.org/10.1002/ana.24999

Evans, G. W. (2004). The Environment of Childhood Poverty. *American Psychologist*, *59*(2), 77–92. https://doi.org/10.1037/0003-066X.59.2.77

Faber, M. J., Bosscher, R. J., Chin A Paw, M. J., & van Wieringen, P. C. (2006). Effects of Exercise Programs on Falls and Mobility in Frail and Pre-Frail Older Adults: A Multicenter Randomized Controlled Trial. *Archives of Physical Medicine and Rehabilitation*, *87*(7), 885–896. https://doi.org/10.1016/j.apmr.2006.04.005

Feng, G., & Zhou, W. (2019). Nostril-specific and structure-based olfactory learning of chiral discrimination in human adults. *ELife*, *8*, e41296. https://doi.org/10.7554/eLife.41296

Fiford, C. M., Ridgway, G. R., Cash, D. M., Modat, M., Nicholas, J., Manning, E. N., Malone, I. B., Biessels, G. J., Ourselin, S., Carmichael, O. T., Cardoso, M. J., & Barnes, J. (2018). Patterns of progressive atrophy vary with age in Alzheimer's disease patients. *Neurobiology of Aging*, *63*, 22–32. https://doi.org/10.1016/j.neurobiolaging.2017.11.002

Firestein, S. (2001). How the olfactory system makes sense of scents. *Nature*, *413*(6852), 211–218. https://doi.org/10.1038/35093026

Fjaeldstad, A., Fernandes, H. M., Van Hartevelt, T. J., Gleesborg, C., Møller, A., Ovesen, T., & Kringelbach, M. L. (2017). Brain fingerprints of olfaction: A novel structural method for assessing olfactory cortical networks in health and disease. *Scientific Reports*, *7*, 42534. https://doi.org/10.1038/srep42534

Fjaeldstad, A. W., Stiller-Stut, F., Gleesborg, C., Kringelbach, M. L., Hummel, T., & Fernandes, H. M. (2021). Validation of Olfactory Network Based on Brain Structural Connectivity and Its Association With Olfactory Test Scores. *Frontiers in Systems Neuroscience*, *15*, 638053. https://doi.org/10.3389/fnsys.2021.638053

Fjell, A. M., & Walhovd, K. B. (2010). Structural Brain Changes in Aging: Courses, Causes and Cognitive Consequences. *Reviews in the Neurosciences*, *21*(3). https://doi.org/10.1515/REVNEURO.2010.21.3.187

Fleisher, A. S., Sun, S., Taylor, C., Ward, C. P., Gamst, A. C., Petersen, R. C., Jack, C. R., Aisen, P. S., & Thal, L. J. (2008). Volumetric MRI vs clinical predictors of Alzheimer disease in mild cognitive impairment. *Neurology*, *70*(3), 191–199. https://doi.org/10.1212/01.wnl.0000287091.57376.65

Fletcher, D. A., & Mullins, R. D. (2010). Cell mechanics and the cytoskeleton. *Nature*, *463*(7280), 485–492. https://doi.org/10.1038/nature08908

Florentin, A., & Arama, E. (2012). Caspase levels and execution efficiencies determine the apoptotic potential of the cell. *The Journal of Cell Biology*, *196*(4), 513–527. https://doi.org/10.1083/jcb.201107133

Flores, J., Noël, A., Foveau, B., Lynham, J., Lecrux, C., & LeBlanc, A. C. (2018). Caspase-1 inhibition alleviates cognitive impairment and neuropathology in an Alzheimer's disease mouse model. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-06449-x

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, *12*(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Fong, T. G., Fearing, M. A., Jones, R. N., Shi, P., Marcantonio, E. R., Rudolph, J. L., Yang, F. M., Dan Kiely, K., & Inouye, S. K. (2009). Telephone Interview for Cognitive Status: Creating a crosswalk with the Mini-Mental State Examination. *Alzheimer's & Dementia*, *5*(6), 492–497. https://doi.org/10.1016/j.jalz.2009.02.007

Fotenos, A. F., Mintun, M. A., Snyder, A. Z., Morris, J. C., & Buckner, R. L. (2008). Brain Volume Decline in Aging: Evidence for a Relation Between Socioeconomic Status, Preclinical Alzheimer Disease, and Reserve. *Archives of Neurology*, *65*(1). https://doi.org/10.1001/archneurol.2007.27

Fournel, A., Sezille, C., Licon, C. C., Sinding, C., Gerber, J., Ferdenzi, C., Hummel, T., & Bensafi, M. (2017). Learning to name smells increases activity in heteromodal semantic areas: Learning to Name Smells. *Human Brain Mapping*, *38*(12), 5958–5969. https://doi.org/10.1002/hbm.23801

Foveau, B., Albrecht, S., Bennett, D. A., Correa, J. A., & LeBlanc, A. C. (2016). Increased Caspase-6 activity in the human anterior olfactory nuclei of the olfactory bulb is associated with cognitive impairment. *Acta Neuropathologica Communications*, *4*(1). https://doi.org/10.1186/s40478-016-0400-x

Franks, K. H., Chuah, M. I., King, A. E., & Vickers, J. C. (2015). Connectivity of Pathology: The Olfactory System as a Model for Network-Driven Mechanisms of Alzheimer's Disease Pathogenesis. *Frontiers in Aging Neuroscience*, *7*. https://doi.org/10.3389/fnagi.2015.00234

Frisoni, G. B., Ganzola, R., Canu, E., Rub, U., Pizzini, F. B., Alessandrini, F., Zoccatelli, G., Beltramello, A., Caltagirone, C., & Thompson, P. M. (2008). Mapping local hippocampal changes in Alzheimer's disease and normal ageing with MRI at 3 Tesla. *Brain*, *131*(12), 3266–3276. https://doi.org/10.1093/brain/awn280

Fusetti, M., Fioretti, A. B., Silvagni, F., Simaskou, M., Sucapane, P., Necozione, S., & Eibenstein, A. (2010). Smell and preclinical Alzheimer disease: Study of 29 patients with amnesic mild cognitive impairment. *Journal of Otolaryngology - Head & Neck Surgery = Le Journal D'oto-Rhino-Laryngologie Et De Chirurgie Cervico-Faciale*, *39*(2), 175–181.

Gao, H.-M., & Hong, J.-S. (2008). Why neurodegenerative diseases are progressive: Uncontrolled inflammation drives disease progression. *Trends in Immunology*, *29*(8), 357–365. https://doi.org/10.1016/j.it.2008.05.002

Garcia-Falgueras, A., Junque, C., Giménez, M., Caldú, X., Segovia, S., & Guillamon, A. (2006). Sex differences in the human olfactory system. *Brain Research*, *1116*(1), 103–111. https://doi.org/10.1016/j.brainres.2006.07.115

Gaudreau, P., Morais, J. A., Shatenstein, B., Gray-Donald, K., Khalil, A., Dionne, I., Ferland, G., Fülöp, T., Jacques, D., Kergoat, M.-J., Tessier, D., Wagner, R., & Payette, H. (2007). Nutrition as a Determinant of Successful Aging: Description of the Quebec Longitudinal Study NuAge and Results from Cross-Sectional Pilot Studies. *Rejuvenation Research*, *10*(3), 377–386. https://doi.org/10.1089/rej.2007.0596

Geroldi, C. (2000). Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer's disease: A volumetric MRI study. *Journal of Neurology, Neurosurgery & Psychiatry*, *68*(1), 93–96. https://doi.org/10.1136/jnnp.68.1.93

Giannakopoulos, P., Kövari, E., Herrmann, F. R., Hof, P. R., & Bouras, C. (2009). Interhemispheric Distribution of Alzheimer Disease and Vascular Pathology in Brain Aging. *Stroke*, *40*(3), 983–986. https://doi.org/10.1161/STROKEAHA.108.530337

Gianotti, L. R. R., Künig, G., Lehmann, D., Faber, P. L., Pascual-Marqui, R. D., Kochi, K., & Schreiter-Gasser, U. (2007). Correlation between disease severity and brain electric LORETA tomography in Alzheimer's disease. *Clinical Neurophysiology*, *118*(1), 186–196. https://doi.org/10.1016/j.clinph.2006.09.007

Gilbert, P. E., Barr, P. J., & Murphy, C. (2004). Differences in olfactory and visual memory in patients with pathologically confirmed Alzheimer's disease and the Lewy body variant of Alzheimer's disease.

Journal of the International Neuropsychological Society, *10*(6), 835–842. https://doi.org/10.1017/S1355617704106024

Gili, T., Cercignani, M., Serra, L., Perri, R., Giove, F., Maraviglia, B., Caltagirone, C., & Bozzali, M. (2011). Regional brain atrophy and functional disconnection across Alzheimer's disease evolution. *Journal of Neurology, Neurosurgery, and Psychiatry*, *82*(1), 58–66. https://doi.org/10.1136/jnnp.2009.199935

Gitler, A. D., Dhillon, P., & Shorter, J. (2017). Neurodegenerative disease: Models, mechanisms, and a new hope. *Disease Models & Mechanisms*, *10*(5), 499–502. https://doi.org/10.1242/dmm.030205

Gobba, F., & Abbacchini, C. (2012). Anosmia after exposure to a pyrethrin-based insecticide: A case report. *International Journal of Occupational Medicine and Environmental Health*, *25*(4), 506–512. https://doi.org/10.2478/S13382-012-0060-4

Godoy, M., Voegels, R., Pinna, F., Imamura, R., & Farfel, J. (2014). Olfaction in Neurologic and Neurodegenerative Diseases: A Literature Review. *International Archives of Otorhinolaryngology*, *19*(02), 176–179. https://doi.org/10.1055/s-0034-1390136

Godyń, J., Jończyk, J., Panek, D., & Malawska, B. (2016). Therapeutic strategies for Alzheimer's disease in clinical trials. *Pharmacological Reports*, *68*(1), 127–138. https://doi.org/10.1016/j.pharep.2015.07.006

Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., & Crowther, R. A. (1989). Multiple isoforms of human microtubule-associated protein tau: Sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*, *3*(4), 519–526. https://doi.org/10.1016/0896-6273(89)90210-9

Gong, C.-X., & Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: A promising therapeutic target for Alzheimer disease. *Current Medicinal Chemistry*, *15*(23), 2321–2328.

Goode, B. L., & Feinstein, S. C. (1994). Identification of a novel microtubule binding and assembly domain in the developmentally regulated inter-repeat region of tau. *The Journal of Cell Biology*, *124*(5), 769–782.

Gottfried, J. A. (2006). Smell: Central Nervous Processing. In T. Hummel & A. Welge-Lüssen (Eds.), *Advances in Oto-Rhino-Laryngology* (Vol. 63, pp. 44–69). KARGER. https://doi.org/10.1159/000093750

Gottfried, J. A., Deichmann, R., Winston, J. S., & Dolan, R. J. (2002). Functional Heterogeneity in Human Olfactory Cortex: An Event-Related Functional Magnetic Resonance Imaging Study. *The Journal of Neuroscience*, *22*(24), 10819–10828. https://doi.org/10.1523/JNEUROSCI.22-24-10819.2002

Graham, R. K., Ehrnhoefer, D. E., & Hayden, M. R. (2011). Caspase-6 and neurodegeneration. *Trends in Neurosciences*, *34*(12), 646–656. https://doi.org/10.1016/j.tins.2011.09.001

Grajauskas, L. A., Siu, W., Medvedev, G., Guo, H., D'Arcy, R. C. N., & Song, X. (2019). MRI-based evaluation of structural degeneration in the ageing brain: Pathophysiology and assessment. *Ageing Research Reviews*, *49*, 67–82. https://doi.org/10.1016/j.arr.2018.11.004

Gray, A. J., Staples, V., Murren, K., Dhariwal, A., & Bentham, P. (2001). Olfactory identification is impaired in clinic-based patients with vascular dementia and senile dementia of Alzheimer type. *International Journal of Geriatric Psychiatry*, *16*(5), 513–517. https://doi.org/10.1002/gps.383

Graziadei, P. P. C., Karlan, M. S., Monti, G. A., & Bernstein, J. J. (1980). Neurogenesis of sensory neurons in the primate olfactory system after section of the fila olfactoria. *Brain Research*, *186*(2), 289–300. https://doi.org/10.1016/0006-8993(80)90976-2

Griffin, M. O., Fricovsky, E., Ceballos, G., & Villarreal, F. (2010). Tetracyclines: A pleitropic family of compounds with promising therapeutic properties. Review of the literature. *American Journal of Physiology-Cell Physiology*, *299*(3), C539–C548. https://doi.org/10.1152/ajpcell.00047.2010

Growdon, M. E., Schultz, A. P., Dagley, A. S., Amariglio, R. E., Hedden, T., Rentz, D. M., Johnson, K. A., Sperling, R. A., Albers, M. W., & Marshall, G. A. (2015). Odor identification and Alzheimer disease biomarkers in clinically normal elderly. *Neurology*, *84*(21), 2153–2160. https://doi.org/10.1212/WNL.0000000000001614

Guerreiro, R., & Bras, J. (2015). The age factor in Alzheimer's disease. *Genome Medicine*, *7*(1), 106. https://doi.org/10.1186/s13073-015-0232-5

Guerreiro, R., Brás, J., & Hardy, J. (2013). SnapShot: Genetics of Alzheimer's Disease. *Cell*, *155*(4), 968-968.e1. https://doi.org/10.1016/j.cell.2013.10.037

Gulland, A. (2012). Number of people with dementia will reach 65.7 million by 2030, says report. *BMJ*, *344*(apr11 1), e2604–e2604. https://doi.org/10.1136/bmj.e2604

Guo, J. Y., Isohanni, M., Miettunen, J., Jääskeläinen, E., Kiviniemi, V., Nikkinen, J., Remes, J., Huhtaniska, S., Veijola, J., Jones, P. B., & Murray, G. K. (2016). Brain structural changes in women and men during midlife. *Neuroscience Letters*, *615*, 107–112. https://doi.org/10.1016/j.neulet.2016.01.007

Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., Khachaturian, A. S., Vergallo, A., Cavedo, E., Snyder, P. J., & Khachaturian, Z. S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*, *141*(7), 1917–1933. https://doi.org/10.1093/brain/awy132

Hanna Al-Shaikh, F. S., Duara, R., Crook, J. E., Lesser, E. R., Schaeverbeke, J., Hinkle, K. M., Ross, O. A., Ertekin-Taner, N., Pedraza, O., Dickson, D. W., Graff-Radford, N. R., & Murray, M. E. (2020).

Selective Vulnerability of the Nucleus Basalis of Meynert Among Neuropathologic Subtypes of Alzheimer Disease. *JAMA Neurology*, *77*(2), 225–233. https://doi.org/10.1001/jamaneurol.2019.3606

Harada, C. N., Natelson Love, M. C., & Triebel, K. L. (2013). Normal Cognitive Aging. *Clinics in Geriatric Medicine*, *29*(4), 737–752. https://doi.org/10.1016/j.cger.2013.07.002

Harada, R., Okamura, N., Furumoto, S., Tago, T., Yanai, K., Arai, H., & Kudo, Y. (2016). Characteristics of Tau and Its Ligands in PET Imaging. *Biomolecules*, *6*(1), 7. https://doi.org/10.3390/biom6010007

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M. L., Pahwa, J. S., Moskvina, V., Dowzell, K., Williams, A., Jones, N., Thomas, C., Stretton, A., Morgan, A. R., Lovestone, S., Powell, J., Proitsi, P., Lupton, M. K., Brayne, C., … Williams, J. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nature Genetics*, *41*(10), 1088–1093. https://doi.org/10.1038/ng.440

Hastie, C., Haw, S., & Pell, J. (2008). Impact of smoking cessation and lifetime exposure on C-reactive protein. *Nicotine & Tobacco Research*, *10*(4), 637–642. https://doi.org/10.1080/14622200801978722

Hatami, A., Monjazeb, S., Milton, S., & Glabe, C. G. (2017). Familial Alzheimer's Disease Mutations within the Amyloid Precursor Protein Alter the Aggregation and Conformation of the Amyloid-β Peptide. *Journal of Biological Chemistry*, *292*(8), 3172–3185. https://doi.org/10.1074/jbc.M116.755264

Hawkes, C. (2003). Olfaction in neurodegenerative disorder. *Movement Disorders*, *18*(4), 364–372. https://doi.org/10.1002/mds.10379

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology*, *80*(19), 1778–1783. https://doi.org/10.1212/WNL.0b013e31828726f5

Hedner, M., Nilsson, L.-G., Olofsson, J. K., Bergman, O., Eriksson, E., Nyberg, L., & Larsson, M. (2010). Age-Related Olfactory Decline is Associated with the BDNF Val66met Polymorphism: Evidence from a Population-Based Study. *Frontiers in Aging Neuroscience*, *2*, 24. https://doi.org/10.3389/fnagi.2010.00024

Heininger, K. (2000). A Unifying Hypothesis of Alzheimer's Disease. IV. Causation and Sequence of Events. *Reviews in the Neurosciences*, *11*(Supplement). https://doi.org/10.1515/REVNEURO.2000.11.S1.213

Heinzel, S., Metzger, F. G., Ehlis, A.-C., Korell, R., Alboji, A., Haeussinger, F. B., Wurster, I., Brockmann, K., Suenkel, U., Eschweiler, G. W., Maetzler, W., Berg, D., & Fallgatter, A. J. (2015). Age and Vascular Burden Determinants of Cortical Hemodynamics Underlying Verbal Fluency. *PLOS ONE*, *10*(9), e0138863. https://doi.org/10.1371/journal.pone.0138863

Heneka, M. T., Carson, M. J., Khoury, J. E., Landreth, G. E., Brosseron, F., Feinstein, D. L., Jacobs, A. H., Wyss-Coray, T., Vitorica, J., Ransohoff, R. M., Herrup, K., Frautschy, S. A., Finsen, B., Brown, G. C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., … Kummer, M. P. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*, *14*(4), 388–405. https://doi.org/10.1016/S1474-4422(15)70016-5

Hennebelle, M., Harbeby, E., Tremblay, S., Chouinard-Watkins, R., Pifferi, F., Plourde, M., Guesnet, P., & Cunnane, S. C. (2015). Challenges to determining whether DHA can protect against age-related cognitive decline. *Clinical Lipidology*, *10*(1), 91–102. https://doi.org/10.2217/clp.14.61

Henriques, A. D., Benedet, A. L., Camargos, E. F., Rosa-Neto, P., & Nóbrega, O. T. (2018). Fluid and imaging biomarkers for Alzheimer's disease: Where we stand and where to head to. *Experimental Gerontology*, *107*, 169–177. https://doi.org/10.1016/j.exger.2018.01.002

Hibar, D. P., Stein, J. L., Renteria, M. E., Arias-Vasquez, A., Arias-Vasquez, A., Jahanshad, N., Toro, R., Wittfeld, K., Abramovic, L., Andersson, M., Aribisala, B. S., Armstrong, N. J., Bernard, M., Bohlken, M. M., Boks, M. P., Bralten, J., Brown, A. A., Mallar Chakravarty, M., Chen, Q., … Medland, S. E. (2015). Common genetic variants influence human subcortical brain structures. *Nature*, *520*(7546), 224–229. https://doi.org/10.1038/nature14101

Hippius, H., & Neundörfer, G. (2003). The discovery of Alzheimer's disease. *Dialogues in Clinical Neuroscience*, *5*(1), 101–108.

Hirai, T., Kojima, S., Shimada, A., Umemura, T., Sakai, M., & Itakurat, C. (1996). Age-related changes in the olfactory system of dogs. *Neuropathology and Applied Neurobiology*, *22*(6), 531–539. https://doi.org/10.1111/j.1365-2990.1996.tb01132.x

Holland, D., Desikan, R. S., Dale, A. M., & McEvoy, L. K. (2013). Higher Rates of Decline for Women and Apolipoprotein E ε4 Carriers. *American Journal of Neuroradiology*, *34*(12), 2287–2293. https://doi.org/10.3174/ajnr.A3601

Hu, W., Wu, F., Zhang, Y., Gong, C.-X., Iqbal, K., & Liu, F. (2017). Expression of Tau Pathology-Related Proteins in Different Brain Regions: A Molecular Basis of Tau Pathogenesis. *Frontiers in Aging Neuroscience*, *9*. https://doi.org/10.3389/fnagi.2017.00311

Huart, C., Rombaux, P., & Hummel, T. (2013). Plasticity of the Human Olfactory System: The Olfactory Bulb. *Molecules*, *18*(9), 11586–11600. https://doi.org/10.3390/molecules180911586

Hugdahl, K. (2005). Symmetry and asymmetry in the human brain. *European Review*, *13*(S2), 119. https://doi.org/10.1017/S1062798705000700

Hummel, T., Rissom, K., Reden, J., Hähner, A., Weidenbecher, M., & Hüttenbrink, K.-B. (2009). Effects of olfactory training in patients with olfactory loss. *The Laryngoscope*, *119*(3), 496–499. https://doi.org/10.1002/lary.20101

Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., & Kobal, G. (1997). "Sniffin" sticks': Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chemical Senses*, *22*(1), 39–52. https://doi.org/10.1093/chemse/22.1.39

Iizuka, N., Masaoka, Y., Kubota, S., Sugiyama, H., Yoshida, M., Yoshikawa, A., Koiwa, N., Honma, M., Watanabe, K., Kamijo, S., Kamimura, S., Ida, M., Ono, K., & Izumizaki, M. (2021). Entorhinal cortex and parahippocampus volume reductions impact olfactory decline in aged subjects. *Brain and Behavior*, *11*(5). https://doi.org/10.1002/brb3.2115

Israel, M. A., Yuan, S. H., Bardy, C., Reyna, S. M., Mu, Y., Herrera, C., Hefferan, M. P., Van Gorp, S., Nazor, K. L., Boscolo, F. S., Carson, C. T., Laurent, L. C., Marsala, M., Gage, F. H., Remes, A. M., Koo, E. H., & Goldstein, L. S. B. (2012). Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature*, *482*(7384), 216–220. https://doi.org/10.1038/nature10821

Iwakiri, M., Mizukami, K., Ishikawa, M., & Asada, T. (2006). GABAA receptor γ subunits in the hippocampus of the rat after perforant pathway lesion. *Neuroscience Letters*, *394*(2), 88–91. https://doi.org/10.1016/j.neulet.2005.10.015

J. Dejerine. (1895). *Anatomie des Centres Nerveux* (Vol. 51). Rueff.

Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., … Silverberg, N. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & Dementia*, *14*(4), 535–562. https://doi.org/10.1016/j.jalz.2018.02.018

Jack, C. R., Knopman, D. S., Jagust, W. J., Shaw, L. M., Aisen, P. S., Weiner, M. W., Petersen, R. C., & Trojanowski, J. Q. (2010). Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet Neurology*, *9*(1), 119–128. https://doi.org/10.1016/S1474-4422(09)70299-6

Jackman, A. H., & Doty, R. L. (2005). Utility of a three-item smell identification test in detecting olfactory dysfunction. *The Laryngoscope*, *115*(12), 2209–2212. https://doi.org/10.1097/01.mlg.0000183194.17484.bb

Jalaluddin, W. M. S., Mat Jusoh, N., Ali Basahai, I. A., Abdullah, M. S., Abdul Karim, A. H., & Gazali, A. K. (2013). Normalised MRI Volumetry of the Hippocampus among Normal Malay Children and Adolescents. *The Malaysian Journal of Medical Sciences: MJMS*, *20*(1), 31–38.

Jernigan, T. L., Archibald, S. L., Fennema-Notestine, C., Gamst, A. C., Stout, J. C., Bonner, J., & Hesselink, J. R. (2001). Effects of age on tissues and regions of the cerebrum and cerebellum. *Neurobiology of Aging*, *22*(4), 581–594. https://doi.org/10.1016/S0197-4580(01)00217-2

Jiang, J., Sachdev, P., Lipnicki, D. M., Zhang, H., Liu, T., Zhu, W., Suo, C., Zhuang, L., Crawford, J., Reppermund, S., Trollor, J., Brodaty, H., & Wen, W. (2014). A longitudinal study of brain atrophy over two years in community-dwelling older individuals. *NeuroImage*, *86*, 203–211. https://doi.org/10.1016/j.neuroimage.2013.08.022

Jimbo, D., Kimura, Y., Taniguchi, M., Inoue, M., & Urakami, K. (2009). Effect of aromatherapy on patients with Alzheimer's disease. *Psychogeriatrics: The Official Journal of the Japanese Psychogeriatric Society*, *9*(4), 173–179. https://doi.org/10.1111/j.1479-8301.2009.00299.x

Johnson, K. A., Fox, N. C., Sperling, R. A., & Klunk, W. E. (2012). Brain Imaging in Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(4), a006213–a006213. https://doi.org/10.1101/cshperspect.a006213

Jouanne, M., Rault, S., & Voisin-Chiret, A.-S. (2017). Tau protein aggregation in Alzheimer's disease: An attractive target for the development of novel therapeutic agents. *European Journal of Medicinal Chemistry*, *139*, 153–167. https://doi.org/10.1016/j.ejmech.2017.07.070

Julien, O., & Wells, J. A. (2017). Caspases and their substrates. *Cell Death & Differentiation*, *24*(8), 1380–1389. https://doi.org/10.1038/cdd.2017.44

Juottonen, K., Laakso, M. P., Insausti, R., Lehtovirta, M., Pitkänen, A., Partanen, K., & Soininen, H. (1998). Volumes of the Entorhinal and Perirhinal Cortices in Alzheimer's Disease. *Neurobiology of Aging*, *19*(1), 15–22. https://doi.org/10.1016/S0197-4580(98)00007-4

Kandimalla, R., & Reddy, P. H. (2017). Therapeutics of Neurotransmitters in Alzheimer's Disease. *Journal of Alzheimer's Disease: JAD*, *57*(4), 1049–1069. https://doi.org/10.3233/JAD-161118

Karch, C. M., & Goate, A. M. (2015). Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. *Biological Psychiatry*, *77*(1), 43–51. https://doi.org/10.1016/j.biopsych.2014.05.006

Karunanayaka, P., Eslinger, P. J., Wang, J.-L., Weitekamp, C. W., Molitoris, S., Gates, K. M., Molenaar, P. C. M., & Yang, Q. X. (2014). Networks involved in olfaction and their dynamics using independent component analysis and unified structural equation modeling: Networks Involved in Olfaction and Their Dynamics. *Human Brain Mapping*, *35*(5), 2055–2072. https://doi.org/10.1002/hbm.22312

Karunanayaka, P., Tobia, M. J., & Yang, Q. X. (2017). Age-related resting-state functional connectivity in the olfactory and trigeminal networks. *Neuroreport*, *28*(15), 943–948. https://doi.org/10.1097/WNR.0000000000000850

Kashibayashi, T., Takahashi, R., Fujita, J., Kamimura, N., Okutani, F., & Kazui, H. (2020). Correlation between regional brain volume and olfactory function in very mild amnestic patients. *Journal of the Neurological Sciences*, *411*, 116686. https://doi.org/10.1016/j.jns.2020.116686

Katotomichelakis, M., Balatsouras, D., Tripsianis, G., Davris, S., Maroudias, N., Danielides, V., & Simopoulos, C. (2007). The effect of smoking on the olfactory function. *Rhinology*, *45*(4), 273–280.

Kelly, D. A., Seidenberg, M., Reiter, K., Nielson, K. A., Woodard, J. L., Smith, J. C., Durgerian, S., & Rao, S. M. (2018). Differential 5-year brain atrophy rates in cognitively declining and stable APOE-ε4 elders. *Neuropsychology*, *32*(6), 647–653. https://doi.org/10.1037/neu0000444

Kennedy, R. E., Williams, C. P., Sawyer, P., Allman, R. M., & Crowe, M. (2014). Comparison of In-Person and Telephone Administration of the Mini-Mental State Examination in the University of Alabama at Birmingham Study of Aging. *Journal of the American Geriatrics Society*, *62*(10), 1928–1932. https://doi.org/10.1111/jgs.13027

Kim, J. Y., Rasheed, A., Yoo, S.-J., Kim, S. Y., Cho, B., Son, G., Yu, S.-W., Chang, K.-A., Suh, Y.-H., & Moon, C. (2018). Distinct amyloid precursor protein processing machineries of the olfactory system. *Biochemical and Biophysical Research Communications*, *495*(1), 533–538. https://doi.org/10.1016/j.bbrc.2017.10.153

Kjelvik, G., Saltvedt, I., White, L. R., Stenumgård, P., Sletvold, O., Engedal, K., Skåtun, K., Lyngvær, A. K., Steffenach, H. A., & Håberg, A. K. (2014). The brain structural and cognitive basis of odor identification deficits in mild cognitive impairment and Alzheimer's disease. *BMC Neurology*, *14*, 168. https://doi.org/10.1186/s12883-014-0168-1

Koca, E., Taşkapilioğlu, Ö., & Bakar, M. (2017). Caregiver Burden in Different Stages of Alzheimer's Disease. *Noro Psikiyatri Arsivi*, *54*(1), 82–86. https://doi.org/10.5152/npa.2017.11304

Kocahan, S., & Doğan, Z. (2017). Mechanisms of Alzheimer's Disease Pathogenesis and Prevention: The Brain, Neural Pathology, N-methyl-D-aspartate Receptors, Tau Protein and Other Risk Factors. *Clinical Psychopharmacology and Neuroscience*, *15*(1), 1–8. https://doi.org/10.9758/cpn.2017.15.1.1

Kollndorfer, K., Kowalczyk, K., Hoche, E., Mueller, C. A., Pollak, M., Trattnig, S., & Schöpf, V. (2014). Recovery of Olfactory Function Induces Neuroplasticity Effects in Patients with Smell Loss. *Neural Plasticity*, *2014*, 1–7. https://doi.org/10.1155/2014/140419

Kondo, K., Kikuta, S., Ueha, R., Suzukawa, K., & Yamasoba, T. (2020). Age-Related Olfactory Dysfunction: Epidemiology, Pathophysiology, and Clinical Management. *Frontiers in Aging Neuroscience*, *12*, 208. https://doi.org/10.3389/fnagi.2020.00208

Kong, V., Devenyi, G. A., Gallino, D., Ayranci, G., Germann, J., Rollins, C., & Chakravarty, M. M. (2018). Early-in-life neuroanatomical and behavioural trajectories in a triple transgenic model of Alzheimer's disease. *Brain Structure & Function*, *223*(7), 3365–3382. https://doi.org/10.1007/s00429-018-1691-4

Kosik, K. S., Orecchio, L. D., Bakalis, S., & Neve, R. L. (1989). Developmentally regulated expression of specific tau sequences. *Neuron*, *2*(4), 1389–1397.

Koss, W. A., & Frick, K. M. (2017). Sex differences in hippocampal function: Sex Differences in Hippocampal Function. *Journal of Neuroscience Research*, *95*(1–2), 539–562. https://doi.org/10.1002/jnr.23864

Köster, S., Weitz, D. A., Goldman, R. D., Aebi, U., & Herrmann, H. (2015). Intermediate filament mechanics in vitro and in the cell: From coiled coils to filaments, fibers and networks. *Current Opinion in Cell Biology*, *32*, 82–91. https://doi.org/10.1016/j.ceb.2015.01.001

Kovács, T., Cairns, N. J., & Lantos, P. L. (2001a). Olfactory centres in Alzheimer's disease: Olfactory bulb is involved in early Braak's stages: *Neuroreport*, *12*(2), 285–288. https://doi.org/10.1097/00001756-200102120-00021

Kovács, T., Cairns, N. J., & Lantos, P. L. (2001b). Olfactory centres in Alzheimer's disease: Olfactory bulb is involved in early Braak's stages. *Neuroreport*, *12*(2), 285–288.

Krajnik, J., Kollndorfer, K., Notter, L. A., Mueller, C. A., & Schöpf, V. (2015). The impact of olfactory dysfunction on interoceptive awareness: Olfactory dysfunction and interoception. *Psychophysiology*, *52*(2), 263–268. https://doi.org/10.1111/psyp.12316

Kramer, A. F., Hahn, S., Cohen, N. J., Banich, M. T., McAuley, E., Harrison, C. R., Chason, J., Vakil, E., Bardell, L., Boileau, R. A., & Colcombe, A. (1999). Ageing, fitness and neurocognitive function. *Nature*, *400*(6743), 418–419. https://doi.org/10.1038/22682

Kubota, S., Masaoka, Y., Sugiyama, H., Yoshida, M., Yoshikawa, A., Koiwa, N., Honma, M., Kinno, R., Watanabe, K., Iizuka, N., Ida, M., Ono, K., & Izumizaki, M. (2020). Hippocampus and Parahippocampus Volume Reduction Associated With Impaired Olfactory Abilities in Subjects Without Evidence of Cognitive Decline. *Frontiers in Human Neuroscience*, *14*, 556519. https://doi.org/10.3389/fnhum.2020.556519

Künzi, V., Glatzel, M., Nakano, M. Y., Greber, U. F., Van Leuven, F., & Aguzzi, A. (2002). Unhampered Prion Neuroinvasion despite Impaired Fast Axonal Transport in Transgenic Mice Overexpressing Four-Repeat Tau. *The Journal of Neuroscience*, *22*(17), 7471–7477. https://doi.org/10.1523/JNEUROSCI.22-17-07471.2002

Lanari, A., Amenta, F., Silvestrelli, G., Tomassoni, D., & Parnetti, L. (2006). Neurotransmitter deficits in behavioural and psychological symptoms of Alzheimer's disease. *Mechanisms of Ageing and Development*, *127*(2), 158–165. https://doi.org/10.1016/j.mad.2005.09.016

Landis, B. N., Hummel, T., Hugentobler, M., Giger, R., & Lacroix, J. S. (2003). Ratings of overall olfactory function. *Chemical Senses*, *28*(8), 691–694. https://doi.org/10.1093/chemse/bjg061

Langlois, F., Vu, T. T. M., Chasse, K., Dupuis, G., Kergoat, M.-J., & Bherer, L. (2013). Benefits of Physical Exercise Training on Cognition and Quality of Life in Frail Older Adults. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *68*(3), 400–404. https://doi.org/10.1093/geronb/gbs069

Langstaff, L., Clark, A., Salam, M., & Philpott, C. M. (2021). Cultural Adaptation and Validity of the Sniffin' Sticks Psychophysical Test for the UK Setting. *Chemosensory Perception*. https://doi.org/10.1007/s12078-021-09287-2

Larson, E. B., Wang, L., Bowen, J. D., McCormick, W. C., Teri, L., Crane, P., & Kukull, W. (2006). Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Annals of Internal Medicine*, *144*(2), 73–81. https://doi.org/10.7326/0003-4819-144-2-200601170-00004

Larsson, M., Hedner, M., Papenberg, G., Seubert, J., Bäckman, L., & Laukka, E. J. (2016a). Olfactory memory in the old and very old: Relations to episodic and semantic memory and APOE genotype. *Neurobiology of Aging*, *38*, 118–126. https://doi.org/10.1016/j.neurobiolaging.2015.11.012

Larsson, M., Hedner, M., Papenberg, G., Seubert, J., Bäckman, L., & Laukka, E. J. (2016b). Olfactory memory in the old and very old: Relations to episodic and semantic memory and APOE genotype. *Neurobiology of Aging*, *38*, 118–126. https://doi.org/10.1016/j.neurobiolaging.2015.11.012

Lautenschlager, N. T., Cox, K. L., Flicker, L., Foster, J. K., van Bockxmeer, F. M., Xiao, J., Greenop, K. R., & Almeida, O. P. (2008). Effect of Physical Activity on Cognitive Function in Older Adults at Risk for

Alzheimer Disease: A Randomized Trial. *JAMA*, *300*(9), 1027. https://doi.org/10.1001/jama.300.9.1027

Lavenex, P., & Amaral, D. G. (2000). Hippocampal-neocortical interaction: A hierarchy of associativity. *Hippocampus*, *10*(4), 420–430. https://doi.org/10.1002/1098-1063(2000)10:4<420::AID-HIPO8>3.0.CO;2-5

Lazarov, O., & Marr, R. A. (2010). Neurogenesis and Alzheimer's disease: At the crossroads. *Experimental Neurology*, *223*(2), 267–281. https://doi.org/10.1016/j.expneurol.2009.08.009

Lazarov, O., & Marr, R. A. (2013). Of mice and men: Neurogenesis, cognition and Alzheimer's disease. *Frontiers in Aging Neuroscience*, *5*. https://doi.org/10.3389/fnagi.2013.00043

LeBlanc, A. C. (2013). Caspase-6 as a novel early target in the treatment of Alzheimer's disease. *European Journal of Neuroscience*, *37*(12), 2005–2018. https://doi.org/10.1111/ejn.12250

Lechien, J. R., Chiesa-Estomba, C. M., De Siati, D. R., Horoi, M., Le Bon, S. D., Rodriguez, A., Dequanter, D., Blecic, S., El Afia, F., Distinguin, L., Chekkoury-Idrissi, Y., Hans, S., Delgado, I. L., Calvo-Henriquez, C., Lavigne, P., Falanga, C., Barillari, M. R., Cammaroto, G., Khalife, M., … Saussez, S. (2020). Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): A multicenter European study. *European Archives of Oto-Rhino-Laryngology*, *277*(8), 2251–2261. https://doi.org/10.1007/s00405-020-05965-1

Lee, D. Y., Lee, W. H., Wee, J. H., & Kim, J.-W. (2014). Prognosis of postviral olfactory loss: Follow-up study for longer than one year. *American Journal of Rhinology & Allergy*, *28*(5), 419–422. https://doi.org/10.2500/ajra.2014.28.4102

Lee, G., Neve, R. L., & Kosik, K. S. (1989). The microtubule binding domain of tau protein. *Neuron*, *2*(6), 1615–1624.

Lee, J. S., Lippman, S. M., Benner, S. E., Lee, J. J., Ro, J. Y., Lukeman, J. M., Morice, R. C., Peters, E. J., Pang, A. C., & Fritsche, H. A. (1994). Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia. *Journal of Clinical Oncology*, *12*(5), 937–945. https://doi.org/10.1200/JCO.1994.12.5.937

Lee, K., Lee, Y. M., Park, J., Lee, B., Moon, E., Jeong, H., Kim, S. Y., Chung, Y., & Kim, J. (2019). Right hippocampus atrophy is independently associated with Alzheimer's disease with psychosis. *Psychogeriatrics*, *19*(2), 105–110. https://doi.org/10.1111/psyg.12369

Lee, Y.-J., Han, S. B., Nam, S.-Y., Oh, K.-W., & Hong, J. T. (2010). Inflammation and Alzheimer's disease. *Archives of Pharmacal Research*, *33*(10), 1539–1556. https://doi.org/10.1007/s12272-010-1006-7

Lessard-Beaudoin, M., M. Gonzales, L., AlOtaibi, M., Chouinard-Watkins, R., Plourde, M., Calon, F., & Graham, R. K. (2021). Diet enriched in omega-3 fatty acids alleviates olfactory system deficits in APOE4 transgenic mice. *European Journal of Neuroscience*, ejn.15472. https://doi.org/10.1111/ejn.15472

Li, W., Howard, J. D., & Gottfried, J. A. (2010). Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease. *Brain*, *133*(9), 2714–2726. https://doi.org/10.1093/brain/awq209

Liang, X., Yin, Z., Liu, R., Zhao, H., Wu, S., Lu, J., Qing, Z., Wei, Y., Yang, Q., Zhu, B., Xu, Y., & Zhang, B. (2018). The Role of MRI Biomarkers and Their Interactions with Cognitive Status and APOE ε4 in Nondemented Elderly Subjects. *Neuro-Degenerative Diseases*, *18*(5–6), 270–280. https://doi.org/10.1159/000495754

Liepelt-Scarfone, I., Gauss, K., Maetzler, W., Müller, K., Bormann, C., Fruhmann Berger, M., Timmers, M., Streffer, J., & Berg, D. (2013). Evaluation of progression markers in the premotor phase of

Parkinson's disease: The progression markers in the premotor phase study. *Neuroepidemiology*, *41*(3–4), 174–182. https://doi.org/10.1159/000353560

Liesinger, A. M., Graff-Radford, N. R., Duara, R., Carter, R. E., Hanna Al-Shaikh, F. S., Koga, S., Hinkle, K. M., DiLello, S. K., Johnson, M. F., Aziz, A., Ertekin-Taner, N., Ross, O. A., Dickson, D. W., & Murray, M. E. (2018). Sex and age interact to determine clinicopathologic differences in Alzheimer's disease. *Acta Neuropathologica*, *136*(6), 873–885. https://doi.org/10.1007/s00401-018-1908-x

Liljeström, M., Hultén, A., Parkkonen, L., & Salmelin, R. (2009). Comparing MEG and fMRI views to naming actions and objects. *Human Brain Mapping*, *30*(6), 1845–1856. https://doi.org/10.1002/hbm.20785

Lin, F.-C., Chuang, Y.-S., Hsieh, H.-M., Lee, T.-C., Chiu, K.-F., Liu, C.-K., & Wu, M.-T. (2015). Early Statin Use and the Progression of Alzheimer Disease: A Total Population-Based Case-Control Study. *Medicine*, *94*(47), e2143. https://doi.org/10.1097/MD.0000000000002143

Lindgren, L., Bergdahl, J., & Nyberg, L. (2016). Longitudinal Evidence for Smaller Hippocampus Volume as a Vulnerability Factor for Perceived Stress. *Cerebral Cortex*, *26*(8), 3527–3533. https://doi.org/10.1093/cercor/bhw154

Lloret, A., Fuchsberger, T., Giraldo, E., & Viña, J. (2015). Molecular mechanisms linking amyloid β toxicity and Tau hyperphosphorylation in Alzheimer's disease. *Free Radical Biology and Medicine*, *83*, 186–191. https://doi.org/10.1016/j.freeradbiomed.2015.02.028

Loewenstein, D. A., Barker, W. W., Chang, J. Y., Apicella, A., Yoshii, F., Kothari, P., Levin, B., & Duara, R. (1989). Predominant left hemisphere metabolic dysfunction in dementia. *Archives of Neurology*, *46*(2), 146–152. https://doi.org/10.1001/archneur.1989.00520380046012

Low, A., Ng, K. P., Chander, R. J., Wong, B., & Kandiah, N. (2019). Association of Asymmetrical White Matter Hyperintensities and Apolipoprotein E4 on Cognitive Impairment. *Journal of Alzheimer's Disease: JAD*, *70*(3), 953–964. https://doi.org/10.3233/JAD-190159

Lu, J., Yang, Q. X., Zhang, H., Eslinger, P. J., Zhang, X., Wu, S., Zhang, B., Zhu, B., & Karunanayaka, P. R. (2019). Disruptions of the olfactory and default mode networks in Alzheimer's disease. *Brain and Behavior*, *9*(7). https://doi.org/10.1002/brb3.1296

Lundström, J. N., Hummel, T., & Olsson, M. J. (2003). Individual differences in sensitivity to the odor of 4,16-androstadien-3-one. *Chemical Senses*, *28*(7), 643–650. https://doi.org/10.1093/chemse/bjg057

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, *19*(3), 1233–1239. https://doi.org/10.1016/S1053-8119(03)00169-1

Malone, I. B., Leung, K. K., Clegg, S., Barnes, J., Whitwell, J. L., Ashburner, J., Fox, N. C., & Ridgway, G. R. (2015). Accurate automatic estimation of total intracranial volume: A nuisance variable with less nuisance. *NeuroImage*, *104*, 366–372. https://doi.org/10.1016/j.neuroimage.2014.09.034

Malpetti, M., Ballarini, T., Presotto, L., Garibotto, V., Tettamanti, M., Perani, D., Alzheimer's Disease Neuroimaging Initiative (ADNI) database;, & Network for Efficiency and Standardization of Dementia Diagnosis (NEST-DD) database. (2017). Gender differences in healthy aging and Alzheimer's Dementia: A ^{18} F-FDG-PET study of brain and cognitive reserve: Gender, Brain Reserve in Aging, and AD. *Human Brain Mapping*, *38*(8), 4212–4227. https://doi.org/10.1002/hbm.23659

Mancini, A., Gaetani, L., Di Gregorio, M., Tozzi, A., Ghiglieri, V., Calabresi, P., & Di Filippo, M. (2017). Hippocampal neuroplasticity and inflammation: Relevance for multiple sclerosis. *Multiple Sclerosis and Demyelinating Disorders*, *2*(1), 2. https://doi.org/10.1186/s40893-017-0019-1

Mandelkow, E. (1998). Tau in Alzheimer's disease. *Trends in Cell Biology*, *8*(11), 425–427. https://doi.org/10.1016/S0962-8924(98)01368-3

Mandrekar-Colucci, S., & Landreth, G. E. (2010). Microglia and Inflammation in Alzheimers Disease. *CNS & Neurological Disorders - Drug Targets*, *9*(2), 156–167. https://doi.org/10.2174/187152710791012071

Mariano, F., Hamerschmidt, R., Soares, C., & Moreira, A. (2018). The Middle Turbinate Resection and Its Repercussion in Olfaction with the University of Pennsylvania Smell Identification Test (UPSIT). *International Archives of Otorhinolaryngology*, *22*(03), 280–283. https://doi.org/10.1055/s-0037-1608679

Mathias, S. R., Knowles, E. E. M., Kent, J. W., McKay, D. R., Curran, J. E., de Almeida, M. A. A., Dyer, T. D., Göring, H. H. H., Olvera, R. L., Duggirala, R., Fox, P. T., Almasy, L., Blangero, J., & Glahn, David. C. (2016). Recurrent major depression and right hippocampal volume: A bivariate linkage and association study: Recurrent Depression and Right Hippocampal Volume. *Human Brain Mapping*, *37*(1), 191–202. https://doi.org/10.1002/hbm.23025

Mazure, C. M., & Swendsen, J. (2016). Sex differences in Alzheimer's disease and other dementias. *The Lancet Neurology*, *15*(5), 451–452. https://doi.org/10.1016/S1474-4422(16)00067-3

McDonald, A. J., & Mott, D. D. (2017). Functional neuroanatomy of amygdalohippocampal interconnections and their role in learning and memory: Amygdalohippocampal Interconnections. *Journal of Neuroscience Research*, *95*(3), 797–820. https://doi.org/10.1002/jnr.23709

McEvoy, L. K., & Brewer, J. B. (2010). Quantitative structural MRI for early detection of Alzheimer's disease. *Expert Review of Neurotherapeutics*, *10*(11), 1675–1688. https://doi.org/10.1586/ern.10.162

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*, *34*(7), 939–939. https://doi.org/10.1212/WNL.34.7.939

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011a). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, *7*(3), 263–269. https://doi.org/10.1016/j.jalz.2011.03.005

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011b). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, *7*(3), 263–269. https://doi.org/10.1016/j.jalz.2011.03.005

Mendiola-Precoma, J., Berumen, L. C., Padilla, K., & Garcia-Alcocer, G. (2016). Therapies for Prevention and Treatment of Alzheimer's Disease. *BioMed Research International*, *2016*, 1–17. https://doi.org/10.1155/2016/2589276

Menni, C., Valdes, A. M., Freidin, M. B., Ganesh, S., El-Sayed Moustafa, J. S., Visconti, A., Hysi, P., Bowyer, R. C. E., Mangino, M., Falchi, M., Wolf, J., Steves, C. J., & Spector, T. D. (2020). *Loss of smell and taste in combination with other symptoms is a strong predictor of COVID-19 infection* [Preprint]. Epidemiology. https://doi.org/10.1101/2020.04.05.20048421

Miller, S. L., Celone, K., DePeau, K., Diamond, E., Dickerson, B. C., Rentz, D., Pihlajamaki, M., & Sperling, R. A. (2008). Age-related memory impairment associated with loss of parietal deactivation but preserved hippocampal activation. *Proceedings of the National Academy of Sciences*, *105*(6), 2181–2186. https://doi.org/10.1073/pnas.0706818105

Mitchell, A. J., & Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia—Meta-analysis of 41 robust inception cohort studies. *Acta Psychiatrica Scandinavica*, *119*(4), 252–265. https://doi.org/10.1111/j.1600-0447.2008.01326.x

Mitolo, M., Stanzani-Maserati, M., Capellari, S., Testa, C., Rucci, P., Poda, R., Oppi, F., Gallassi, R., Sambati, L., Rizzo, G., Parchi, P., Evangelisti, S., Talozzi, L., Tonon, C., Lodi, R., & Liguori, R. (2019). Predicting conversion from mild cognitive impairment to Alzheimer's disease using brain 1H-MRS and volumetric changes: A two- year retrospective follow-up study. *NeuroImage: Clinical*, *23*, 101843. https://doi.org/10.1016/j.nicl.2019.101843

Möller, H. J., & Graeber, M. B. (1998). The case described by Alois Alzheimer in 1911. Historical and conceptual perspectives based on the clinical record and neurohistological sections. *European Archives of Psychiatry and Clinical Neuroscience*, *248*(3), 111–122.

Moreno-Jiménez, E. P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., Ávila, J., & Llorens-Martín, M. (2019). Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nature Medicine*, *25*(4), 554–560. https://doi.org/10.1038/s41591-019-0375-9

Morley, J. E., Farr, S. A., & Nguyen, A. D. (2018). Alzheimer Disease. *Clinics in Geriatric Medicine*, *34*(4), 591–601. https://doi.org/10.1016/j.cger.2018.06.006

Morris, J. C., Storandt, M., Miller, J. P., McKeel, D. W., Price, J. L., Rubin, E. H., & Berg, L. (2001). Mild cognitive impairment represents early-stage Alzheimer disease. *Archives of Neurology*, *58*(3), 397–405.

Mortby, M. E., Burns, R., Janke, A. L., Sachdev, P. S., Anstey, K. J., & Cherbuin, N. (2014). Relating Education, Brain Structure, and Cognition: The Role of Cardiovascular Disease Risk Factors. *BioMed Research International*, *2014*, 1–13. https://doi.org/10.1155/2014/271487

Mortimer, J. A., Van Duijn, C. M., Chandra, V., Fratiglioni, L., Graves, A. B., Heyman, A., Jorm, A. F., Kokmen, E., Kondo, K., Rocca, W. A., Shalat, S. L., Soininen, H., & A Hofman for the Eurodem Risk Factors Research Group. (1991). Head Trauma as a Risk Factor for Alzheimer's Disease: A Collaborative Re-Analysis of Case-Control Studies. *International Journal of Epidemiology*, *20*(Supplement 2), S28–S35. https://doi.org/10.1093/ije/20.Supplement_2.S28

Mota, S. I., Costa, R. O., Ferreira, I. L., Santana, I., Caldeira, G. L., Padovano, C., Fonseca, A. C., Baldeiras, I., Cunha, C., Letra, L., Oliveira, C. R., Pereira, C. M. F., & Rego, A. C. (2015). Oxidative stress involving changes in Nrf2 and ER stress in early stages of Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1852*(7), 1428–1441. https://doi.org/10.1016/j.bbadis.2015.03.015

Mueller, C. A., & Hummel, T. (2009). Recovery of olfactory function after nine years of post-traumatic anosmia: A case report. *Journal of Medical Case Reports*, *3*(1), 9283. https://doi.org/10.4076/1752-1947-3-9283

Mueller, S. G., Schuff, N., Yaffe, K., Madison, C., Miller, B., & Weiner, M. W. (2010). Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. *Human Brain Mapping*, *31*(9), 1339–1347. https://doi.org/10.1002/hbm.20934

Mukherjee, J., Christian, B. T., Dunigan, K. A., Shi, B., Narayanan, T. K., Satter, M., & Mantil, J. (2002). Brain imaging of 18F-fallypride in normal volunteers: Blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse (New York, N.Y.)*, *46*(3), 170–188. https://doi.org/10.1002/syn.10128

Mullol, J., Alobid, I., Mariño-Sánchez, F., Quintó, L., de Haro, J., Bernal-Sprekelsen, M., Valero, A., Picado, C., & Marin, C. (2012). Furthering the understanding of olfaction, prevalence of loss of smell and risk factors: A population-based survey (OLFACAT study). *BMJ Open*, *2*(6), e001256. https://doi.org/10.1136/bmjopen-2012-001256

Muratore, C. R., Rice, H. C., Srikanth, P., Callahan, D. G., Shin, T., Benjamin, L. N. P., Walsh, D. M., Selkoe, D. J., & Young-Pearse, T. L. (2014). The familial Alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. *Human Molecular Genetics*, *23*(13), 3523–3536. https://doi.org/10.1093/hmg/ddu064

Murphy, C. (2002). Prevalence of Olfactory Impairment in Older Adults. *JAMA*, *288*(18), 2307. https://doi.org/10.1001/jama.288.18.2307

Murphy, C. (2019). Olfactory and other sensory impairments in Alzheimer disease. *Nature Reviews Neurology*, *15*(1), 11–24. https://doi.org/10.1038/s41582-018-0097-5

Murphy, C., Jernigan, T. L., & Fennema-Notestine, C. (2003). Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: A structural MRI study. *Journal of the International Neuropsychological Society: JINS*, *9*(3), 459–471. https://doi.org/10.1017/S1355617703930116

Murphy, C., Nordin, S., & Jinich, S. (1999). Very Early Decline in Recognition Memory for Odors in Alzheimer's Disease. *Aging, Neuropsychology, and Cognition*, *6*(3), 229–240. https://doi.org/10.1076/anec.6.3.229.777

Murphy, C., Schubert, C. R., Cruickshanks, K. J., Klein, B. E. K., Klein, R., & Nondahl, D. M. (2002). Prevalence of olfactory impairment in older adults. *JAMA*, *288*(18), 2307–2312. https://doi.org/10.1001/jama.288.18.2307

Murphy, M. P., & LeVine, H. (2010). Alzheimer's Disease and the Amyloid-β Peptide. *Journal of Alzheimer's Disease*, *19*(1), 311–323. https://doi.org/10.3233/JAD-2010-1221

Murray, M. E., Graff-Radford, N. R., Ross, O. A., Petersen, R. C., Duara, R., & Dickson, D. W. (2011). Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: A retrospective study. *The Lancet. Neurology*, *10*(9), 785–796. https://doi.org/10.1016/S1474-4422(11)70156-9

Nam, E., Nam, G., & Lim, M. H. (2020). Synaptic Copper, Amyloid-β, and Neurotransmitters in Alzheimer's Disease. *Biochemistry*, *59*(1), 15–17. https://doi.org/10.1021/acs.biochem.9b00775

Naya, Y. (2016). Declarative association in the perirhinal cortex. *Neuroscience Research*, *113*, 12–18. https://doi.org/10.1016/j.neures.2016.07.001

Nebel, R. A., Aggarwal, N. T., Barnes, L. L., Gallagher, A., Goldstein, J. M., Kantarci, K., Mallampalli, M. P., Mormino, E. C., Scott, L., Yu, W. H., Maki, P. M., & Mielke, M. M. (2018). Understanding the impact of sex and gender in Alzheimer's disease: A call to action. *Alzheimer's & Dementia*, *14*(9), 1171–1183. https://doi.org/10.1016/j.jalz.2018.04.008

Newkirk, L. A., Kim, J. M., Thompson, J. M., Tinklenberg, J. R., Yesavage, J. A., & Taylor, J. L. (2004). Validation of a 26-Point Telephone Version of the Mini-Mental State Examination. *Journal of Geriatric Psychiatry and Neurology*, *17*(2), 81–87. https://doi.org/10.1177/0891988704264534

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., Bäckman, L., Hänninen, T., Jula, A., Laatikainen, T., Lindström, J., Mangialasche, F., Paajanen, T., Pajala, S., Peltonen, M., Rauramaa, R., Stigsdotter-Neely, A., Strandberg, T., Tuomilehto, J., … Kivipelto, M. (2015). A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): A randomised controlled trial. *The Lancet*, *385*(9984), 2255–2263. https://doi.org/10.1016/S0140-6736(15)60461-5

Niccoli, T., & Partridge, L. (2012). Ageing as a Risk Factor for Disease. *Current Biology*, *22*(17), R741–R752. https://doi.org/10.1016/j.cub.2012.07.024

Niikura, T., Tajima, H., & Kita, Y. (2006). Neuronal cell death in Alzheimer's disease and a neuroprotective factor, humanin. *Current Neuropharmacology*, *4*(2), 139–147.

Nobis, L., Manohar, S. G., Smith, S. M., Alfaro-Almagro, F., Jenkinson, M., Mackay, C. E., & Husain, M. (2019). Hippocampal volume across age: Nomograms derived from over 19,700 people in UK Biobank. *NeuroImage: Clinical*, *23*, 101904. https://doi.org/10.1016/j.nicl.2019.101904

Noble, K. G., Grieve, S. M., Korgaonkar, M. S., Engelhardt, L. E., Griffith, E. Y., Williams, L. M., & Brickman, A. M. (2012). Hippocampal volume varies with educational attainment across the life-span. *Frontiers in Human Neuroscience*, *6*, 307. https://doi.org/10.3389/fnhum.2012.00307

Nordin, S., & Brämerson, A. (2008). Complaints of olfactory disorders: Epidemiology, assessment and clinical implications. *Current Opinion in Allergy and Clinical Immunology*, *8*(1), 10–15. https://doi.org/10.1097/ACI.0b013e3282f3f473

Nordin, S., Brämerson, A., Lidén, E., & Bende, M. (1998). The Scandinavian Odor-Identification Test: Development, reliability, validity and normative data. *Acta Oto-Laryngologica*, *118*(2), 226–234. https://doi.org/10.1080/00016489850154946

Nordin, S., Monsch, A. U., & Murphy, C. (1995). Unawareness of smell loss in normal aging and Alzheimer's disease: Discrepancy between self-reported and diagnosed smell sensitivity. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *50*(4), P187-192. https://doi.org/10.1093/geronb/50b.4.p187

Ohm, T. G., & Braak, H. (1987). Olfactory bulb changes in Alzheimer's disease. *Acta Neuropathologica*, *73*(4), 365–369. https://doi.org/10.1007/BF00688261

Ohsawa, K., Imai, Y., Sasaki, Y., & Kohsaka, S. (2004). Microglia/macrophage-specific protein Iba1 binds to fimbrin and enhances its actin-bundling activity: Fimbrin as an Iba1-interacting molecule. *Journal of Neurochemistry*, *88*(4), 844–856. https://doi.org/10.1046/j.1471-4159.2003.02213.x

Oleson, S., & Murphy, C. (2015). Olfactory Dysfunction in ApoE ε4/4 Homozygotes with Alzheimer's Disease. *Journal of Alzheimer's Disease*, *46*(3), 791–803. https://doi.org/10.3233/JAD-150089

Oliveira-Pinto, A. V., Santos, R. M., Coutinho, R. A., Oliveira, L. M., Santos, G. B., Alho, A. T. L., Leite, R. E. P., Farfel, J. M., Suemoto, C. K., Grinberg, L. T., Pasqualucci, C. A., Jacob-Filho, W., & Lent, R. (2014). Sexual Dimorphism in the Human Olfactory Bulb: Females Have More Neurons and Glial Cells than Males. *PLoS ONE*, *9*(11), e111733. https://doi.org/10.1371/journal.pone.0111733

Olofsson, J. K., Ekström, I., Larsson, M., & Nordin, S. (2021). Olfaction and Aging: A Review of the Current State of Research and Future Directions. *I-Perception*, *12*(3), 204166952110203. https://doi.org/10.1177/20416695211020331

Olofsson, J. K., Josefsson, M., Ekström, I., Wilson, D., Nyberg, L., Nordin, S., Nordin Adolfsson, A., Adolfsson, R., Nilsson, L.-G., & Larsson, M. (2016). Long-term episodic memory decline is associated with olfactory deficits only in carriers of ApoE-ε4. *Neuropsychologia*, *85*, 1–9. https://doi.org/10.1016/j.neuropsychologia.2016.03.004

Orihashi, R., Mizoguchi, Y., Imamura, Y., Yamada, S., Ueno, T., & Monji, A. (2020). Oxytocin and elderly MRI-based hippocampus and amygdala volume: A 7-year follow-up study. *Brain Communications*, *2*(2), fcaa081. https://doi.org/10.1093/braincomms/fcaa081

Ott, A., van Rossum, C. T. M., van Harskamp, F., van de Mheen, H., Hofman, A., & Breteler, M. M. B. (1999). Education and the incidence of dementia in a large population-based study: The Rotterdam Study. *Neurology*, *52*(3), 663–663. https://doi.org/10.1212/WNL.52.3.663

Pachana, N. A., Alpass, F. M., Blakey, J. A., & Long, N. R. (2006). A comparison of the MMSE and the TICS-m in hearing-impaired older adults. *Australasian Journal on Ageing*, *25*(2), 89–93. https://doi.org/10.1111/j.1741-6612.2006.00156.x

Pakkenberg, B. (2003). Aging and the human neocortex. *Experimental Gerontology*, *38*(1–2), 95–99. https://doi.org/10.1016/S0531-5565(02)00151-1

Parsons, C. G., Danysz, W., Dekundy, A., & Pulte, I. (2013). Memantine and Cholinesterase Inhibitors: Complementary Mechanisms in the Treatment of Alzheimer's Disease. *Neurotoxicity Research*, *24*(3), 358–369. https://doi.org/10.1007/s12640-013-9398-z

Pearson, R. C., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., & Powell, T. P. (1985a). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(13), 4531–4534.

Pearson, R. C., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., & Powell, T. P. (1985b). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(13), 4531–4534.

Pedraza, O., Bowers, D., & Gilmore, R. (2004). Asymmetry of the hippocampus and amygdala in MRI volumetric measurements of normal adults. *Journal of the International Neuropsychological Society*, *10*(5), 664–678. https://doi.org/10.1017/S1355617704105080

Peila, R., Rodriguez, B. L., & Launer, L. J. (2002). Type 2 Diabetes, APOE Gene, and the Risk for Dementia and Related Pathologies: The Honolulu-Asia Aging Study. *Diabetes*, *51*(4), 1256–1262. https://doi.org/10.2337/diabetes.51.4.1256

Pekala, K., Chandra, R. K., & Turner, J. H. (2016). Efficacy of olfactory training in patients with olfactory loss: A systematic review and meta-analysis. *International Forum of Allergy & Rhinology*, *6*(3), 299–307. https://doi.org/10.1002/alr.21669

Peng, G.-P., Feng, Z., He, F.-P., Chen, Z.-Q., Liu, X.-Y., Liu, P., & Luo, B.-Y. (2015). Correlation of Hippocampal Volume and Cognitive Performances in Patients with Either Mild Cognitive Impairment or Alzheimer's disease. *CNS Neuroscience & Therapeutics*, *21*(1), 15–22. https://doi.org/10.1111/cns.12317

Perl, D. P. (2010). Neuropathology of Alzheimer's Disease. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, *77*(1), 32–42. https://doi.org/10.1002/msj.20157

Peters, A. (1998). Feature article: Are neurons lost from the primate cerebral cortex during normal aging? *Cerebral Cortex*, *8*(4), 295–300. https://doi.org/10.1093/cercor/8.4.295

Peters, J. M., Hummel, T., Kratzsch, T., Lötsch, J., Skarke, C., & Frölich, L. (2003). Olfactory Function in Mild Cognitive Impairment and Alzheimer's Disease: An Investigation Using Psychophysical and Electrophysiological Techniques. *American Journal of Psychiatry*, *160*(11), 1995–2002. https://doi.org/10.1176/appi.ajp.160.11.1995

Peters, R. (2006). Ageing and the brain. *Postgraduate Medical Journal*, *82*(964), 84–88. https://doi.org/10.1136/pgmj.2005.036665

Petersen, R. C. (2000). Mild cognitive impairment: Transition between aging and Alzheimer's disease. *Neurologia (Barcelona, Spain)*, *15*(3), 93–101.

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. *Journal of Internal Medicine*, *256*(3), 183–194. https://doi.org/10.1111/j.1365-2796.2004.01388.x

Petersen, R. C., Roberts, R. O., Knopman, D. S., Boeve, B. F., Geda, Y. E., Ivnik, R. J., Smith, G. E., & Jack, C. R. (2009). Mild Cognitive Impairment: Ten Years Later. *Archives of Neurology*, *66*(12). https://doi.org/10.1001/archneurol.2009.266

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., & Kokmen, E. (1999). Mild Cognitive Impairment: Clinical Characterization and Outcome. *Archives of Neurology*, *56*(3), 303. https://doi.org/10.1001/archneur.56.3.303

Picillo, M., Pellecchia, M. T., Erro, R., Amboni, M., Vitale, C., Iavarone, A., Moccia, M., Allocca, R., Orefice, G., & Barone, P. (2014). The use of University of Pennsylvania Smell Identification Test in the diagnosis of Parkinson's disease in Italy. *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *35*(3), 379–383. https://doi.org/10.1007/s10072-013-1522-6

Pievani, M., Galluzzi, S., Thompson, P. M., Rasser, P. E., Bonetti, M., & Frisoni, G. B. (2011). APOE4 is associated with greater atrophy of the hippocampal formation in Alzheimer's disease. *NeuroImage*, *55*(3), 909–919. https://doi.org/10.1016/j.neuroimage.2010.12.081

Pini, L., Pievani, M., Bocchetta, M., Altomare, D., Bosco, P., Cavedo, E., Galluzzi, S., Marizzoni, M., & Frisoni, G. B. (2016). Brain atrophy in Alzheimer's Disease and aging. *Ageing Research Reviews*, *30*, 25–48. https://doi.org/10.1016/j.arr.2016.01.002

Pinto, J. M., Wroblewski, K. E., Kern, D. W., Schumm, L. P., & McClintock, M. K. (2014). Olfactory Dysfunction Predicts 5-Year Mortality in Older Adults. *PLoS ONE*, *9*(10), e107541. https://doi.org/10.1371/journal.pone.0107541

Poellinger, A., Thomas, R., Lio, P., Lee, A., Makris, N., Rosen, B. R., & Kwong, K. K. (2001). Activation and Habituation in Olfaction—An fMRI Study. *NeuroImage*, *13*(4), 547–560. https://doi.org/10.1006/nimg.2000.0713

Poo, M. (2001). Neurotrophins as synaptic modulators. *Nature Reviews Neuroscience*, *2*(1), 24–32. https://doi.org/10.1038/35049004

Pope, S. K., Shue, V. M., & Beck, C. (2003). Will a Healthy Lifestyle Help Prevent Alzheimer's Disease? *Annual Review of Public Health*, *24*(1), 111–132. https://doi.org/10.1146/annurev.publhealth.24.100901.141015

Price, J. L. (1985). Beyond the primary olfactory cortex: Olfactory-related areas in the neocortex, thalamus and hypothalamus. *Chemical Senses*, *10*(2), 239–258. https://doi.org/10.1093/chemse/10.2.239

Price, J. L., Davis, P. B., Morris, J. C., & White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiology of Aging*, *12*(4), 295–312. https://doi.org/10.1016/0197-4580(91)90006-6

Quarmley, M., Moberg, P. J., Mechanic-Hamilton, D., Kabadi, S., Arnold, S. E., Wolk, D. A., & Roalf, D. R. (2016). Odor Identification Screening Improves Diagnostic Classification in Incipient Alzheimer's Disease. *Journal of Alzheimer's Disease*, *55*(4), 1497–1507. https://doi.org/10.3233/JAD-160842

Radak, Z., Hart, N., Sarga, L., Koltai, E., Atalay, M., Ohno, H., & Boldogh, I. (2010). Exercise plays a preventive role against Alzheimer's disease. *Journal of Alzheimer's Disease: JAD*, *20*(3), 777–783. https://doi.org/10.3233/JAD-2010-091531

Rahayel, S., Frasnelli, J., & Joubert, S. (2012). The effect of Alzheimer's disease and Parkinson's disease on olfaction: A meta-analysis. *Behavioural Brain Research*, *231*(1), 60–74. https://doi.org/10.1016/j.bbr.2012.02.047

Ramcharitar, J., M. Afonso, V., Albrecht, S., A. Bennett, D., & LeBlanc, A. C. (2013). Caspase-6 activity predicts lower episodic memory ability in aged individuals. *Neurobiology of Aging*, *34*(7), 1815–1824. https://doi.org/10.1016/j.neurobiolaging.2013.01.007

Rashid, R. A., Zgair, A., & Al-Ani, R. M. (2021). Effect of nasal corticosteroid in the treatment of anosmia due to COVID-19: A randomised double-blind placebo-controlled study. *American Journal of Otolaryngology*, *42*(5), 103033. https://doi.org/10.1016/j.amjoto.2021.103033

Rawal, S., Hoffman, H. J., Chapo, A. K., & Duffy, V. B. (2014). Sensitivity and Specificity of Self-Reported Olfactory Function in a Home-Based Study of Independent-Living, Healthy Older Women. *Chemosensory Perception*, *7*(3–4), 108–116. https://doi.org/10.1007/s12078-014-9170-7

Rawal, S., Hoffman, H. J., Honda, M., Huedo-Medin, T. B., & Duffy, V. B. (2015). The Taste and Smell Protocol in the 2011-2014 US National Health and Nutrition Examination Survey (NHANES): Test-Retest Reliability and Validity Testing. *Chemosensory Perception*, *8*(3), 138–148. https://doi.org/10.1007/s12078-015-9194-7

Rawson, N. E. (2006). Olfactory Loss in Aging. *Science of Aging Knowledge Environment*, *2006*(5), pe6–pe6. https://doi.org/10.1126/sageke.2006.5.pe6

Rawson, N. E., Gomez, G., Cowart, B. J., Kriete, A., Pribitkin, E., & Restrepo, D. (2012). Age-associated loss of selectivity in human olfactory sensory neurons. *Neurobiology of Aging*, *33*(9), 1913–1919. https://doi.org/10.1016/j.neurobiolaging.2011.09.036

Raz, N., Gunning-Dixon, F., Head, D., Rodrigue, K. M., Williamson, A., & Acker, J. D. (2004). Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: Replicability of regional

differences in volume. *Neurobiology of Aging*, *25*(3), 377–396. https://doi.org/10.1016/S0197-4580(03)00118-0

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., Dahle, C., Gerstorf, D., & Acker, J. D. (2005). Regional Brain Changes in Aging Healthy Adults: General Trends, Individual Differences and Modifiers. *Cerebral Cortex*, *15*(11), 1676–1689. https://doi.org/10.1093/cercor/bhi044

Reiter, K., Nielson, K. A., Durgerian, S., Woodard, J. L., Smith, J. C., Seidenberg, M., Kelly, D. A., & Rao, S. M. (2016). Five-Year Longitudinal Brain Volume Change in Healthy Elders at Genetic Risk for Alzheimer's Disease. *Journal of Alzheimer's Disease*, *55*(4), 1363–1377. https://doi.org/10.3233/JAD-160504

Rey, N. L., Wesson, D. W., & Brundin, P. (2018). The olfactory bulb as the entry site for prion-like propagation in neurodegenerative diseases. *Neurobiology of Disease*, *109*, 226–248. https://doi.org/10.1016/j.nbd.2016.12.013

Reynolds, G. P., Mason, S. L., Meldrum, A., Keczer, S., Parties, H., Eglen, R. M., & Wong, E. H. F. (1995). 5-Hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: Distribution, pharmacology and effects of neurodegenerative diseases. *British Journal of Pharmacology*, *114*(5), 993–998. https://doi.org/10.1111/j.1476-5381.1995.tb13303.x

Richard L Doty. (1995). *The Smell Identification Test™ administration manual* (3rd ed.). Sensonics, Inc., Haddon Heights, NJ.

Riechers, S.-P., Butland, S., Deng, Y., Skotte, N., Ehrnhoefer, D. E., Russ, J., Laine, J., Laroche, M., Pouladi, M. A., Wanker, E. E., Hayden, M. R., & Graham, R. K. (2016). Interactome network analysis identifies multiple caspase-6 interactors involved in the pathogenesis of HD. *Human Molecular Genetics*, *25*(8), 1600–1618. https://doi.org/10.1093/hmg/ddw036

Ritchie, S. J., Dickie, D. A., Cox, S. R., Valdes Hernandez, M. D. C., Corley, J., Royle, N. A., Pattie, A., Aribisala, B. S., Redmond, P., Muñoz Maniega, S., Taylor, A. M., Sibbett, R., Gow, A. J., Starr, J. M., Bastin, M. E., Wardlaw, J. M., & Deary, I. J. (2015). Brain volumetric changes and cognitive ageing during the eighth decade of life. *Human Brain Mapping*, *36*(12), 4910–4925. https://doi.org/10.1002/hbm.22959

Roberts, R. O., Christianson, T. J. H., Kremers, W. K., Mielke, M. M., Machulda, M. M., Vassilaki, M., Alhurani, R. E., Geda, Y. E., Knopman, D. S., & Petersen, R. C. (2016). Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurology*, *73*(1), 93. https://doi.org/10.1001/jamaneurol.2015.2952

Rodriguez, G. A., Burns, M. P., Weeber, E. J., & Rebeck, G. W. (2013). Young APOE4 targeted replacement mice exhibit poor spatial learning and memory, with reduced dendritic spine density in the medial entorhinal cortex. *Learning & Memory*, *20*(5), 256–266. https://doi.org/10.1101/lm.030031.112

Rodríguez, J. J., Noristani, H. N., & Verkhratsky, A. (2012). The serotonergic system in ageing and Alzheimer's disease. *Progress in Neurobiology*, *99*(1), 15–41. https://doi.org/10.1016/j.pneurobio.2012.06.010

Rolls, E. T. (2001). The Rules of Formation of the Olfactory Representations Found in the Orbitofrontal Cortex Olfactory Areas in Primates. *Chemical Senses*, *26*(5), 595–604. https://doi.org/10.1093/chemse/26.5.595

Rolls, E. T. (2019). The orbitofrontal cortex and emotion in health and disease, including depression. *Neuropsychologia*, *128*, 14–43. https://doi.org/10.1016/j.neuropsychologia.2017.09.021

Rolls, E. T., Kringelbach, M. L., & de Araujo, I. E. T. (2003). Different representations of pleasant and unpleasant odours in the human brain. *European Journal of Neuroscience*, *18*(3), 695–703. https://doi.org/10.1046/j.1460-9568.2003.02779.x

Rouby, C., Thomas-Danguin, T., Vigouroux, M., Ciuperca, G., Jiang, T., Alexanian, J., Barges, M., Gallice, I., Degraix, J.-L., & Sicard, G. (2011). The Lyon Clinical Olfactory Test: Validation and Measurement of Hyposmia and Anosmia in Healthy and Diseased Populations. *International Journal of Otolaryngology*, *2011*, 1–9. https://doi.org/10.1155/2011/203805

Ruan, Y., Zheng, X.-Y., Zhang, H.-L., Zhu, W., & Zhu, J. (2012). Olfactory dysfunctions in neurodegenerative disorders. *Journal of Neuroscience Research*, *90*(9), 1693–1700. https://doi.org/10.1002/jnr.23054

Sabia, S., Elbaz, A., Dugravot, A., Head, J., Shipley, M., Hagger-Johnson, G., Kivimaki, M., & Singh-Manoux, A. (2012). Impact of Smoking on Cognitive Decline in Early Old Age: The Whitehall II Cohort Study. *Archives of General Psychiatry*, *69*(6). https://doi.org/10.1001/archgenpsychiatry.2011.2016

Sabine, D., Christian, S., Christian, G., Nicholas, M., Milan, A., Alexander, K., Claus, Z., Afra, W., & Mark, M. (2011). Grey-Matter Atrophy in Alzheimer's Disease is Asymmetric but not Lateralized. *Journal of Alzheimer's Disease*, *2*, 347–357. https://doi.org/10.3233/JAD-2011-110041

Saive, A.-L., Royet, J.-P., & Plailly, J. (2014). A review on the neural bases of episodic odor memory: From laboratory-based to autobiographical approaches. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00240

Salthouse, T. A. (2009). When does age-related cognitive decline begin? *Neurobiology of Aging*, *30*(4), 507–514. https://doi.org/10.1016/j.neurobiolaging.2008.09.023

Santos, D. V., Reiter, E. R., DiNardo, L. J., & Costanzo, R. M. (2004). Hazardous Events Associated With Impaired Olfactory Function. *Archives of Otolaryngology–Head & Neck Surgery*, *130*(3), 317. https://doi.org/10.1001/archotol.130.3.317

Sarafoleanu, C., Mella, C., Georgescu, M., & Perederco, C. (2009). The importance of the olfactory sense in the human behavior and evolution. *Journal of Medicine and Life*, *2*(2), 196–198.

Savelieff, M. G., Nam, G., Kang, J., Lee, H. J., Lee, M., & Lim, M. H. (2019). Development of Multifunctional Molecules as Potential Therapeutic Candidates for Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis in the Last Decade. *Chemical Reviews*, *119*(2), 1221–1322. https://doi.org/10.1021/acs.chemrev.8b00138

Scahill, R. I., Frost, C., Jenkins, R., Whitwell, J. L., Rossor, M. N., & Fox, N. C. (2003a). A Longitudinal Study of Brain Volume Changes in Normal Aging Using Serial Registered Magnetic Resonance Imaging. *Archives of Neurology*, *60*(7), 989. https://doi.org/10.1001/archneur.60.7.989

Scahill, R. I., Frost, C., Jenkins, R., Whitwell, J. L., Rossor, M. N., & Fox, N. C. (2003b). A Longitudinal Study of Brain Volume Changes in Normal Aging Using Serial Registered Magnetic Resonance Imaging. *Archives of Neurology*, *60*(7), 989. https://doi.org/10.1001/archneur.60.7.989

Schiffman, S. (2002). Taste, smell and neuropsychological performance of individuals at familial risk for Alzheimer's disease. *Neurobiology of Aging*, *23*(3), 397–404. https://doi.org/10.1016/S0197-4580(01)00337-2

Schiffman, S. S. (1993). Perception of taste and smell in elderly persons. *Critical Reviews in Food Science and Nutrition*, *33*(1), 17–26. https://doi.org/10.1080/10408399309527608

Schmand, B., Lindeboom, J., Hooijer, C., & Jonker, C. (1995). Relation between education and dementia: The role of test bias revisited. *Journal of Neurology, Neurosurgery, and Psychiatry*, *59*(2), 170–174. https://doi.org/10.1136/jnnp.59.2.170

Schott, J. M., Price, S. L., Frost, C., Whitwell, J. L., Rossor, M. N., & Fox, N. C. (2005). Measuring atrophy in Alzheimer disease: A serial MRI study over 6 and 12 months. *Neurology*, *65*(1), 119–124. https://doi.org/10.1212/01.wnl.0000167542.89697.0f

Schuff, N., Tosun, D., Insel, P. S., Chiang, G. C., Truran, D., Aisen, P. S., Jack, C. R., & Weiner, M. W. (2012). Nonlinear time course of brain volume loss in cognitively normal and impaired elders. *Neurobiology of Aging*, *33*(5), 845–855. https://doi.org/10.1016/j.neurobiolaging.2010.07.012

Sele, S., Liem, F., Mérillat, S., & Jäncke, L. (2020). Decline Variability of Cortical and Subcortical Regions in Aging: A Longitudinal Study. *Frontiers in Human Neuroscience*, *14*, 363. https://doi.org/10.3389/fnhum.2020.00363

Sele, S., Liem, F., Mérillat, S., & Jäncke, L. (2021). Age-related decline in the brain: A longitudinal study on inter-individual variability of cortical thickness, area, volume, and cognition. *NeuroImage*, *240*, 118370. https://doi.org/10.1016/j.neuroimage.2021.118370

Seligman, S. C., Kamath, V., Giovannetti, T., Arnold, S. E., & Moberg, P. J. (2013). Olfaction and apathy in Alzheimer's disease, mild cognitive impairment, and healthy older adults. *Aging & Mental Health*, *17*(5), 564–570. https://doi.org/10.1080/13607863.2013.768208

Sengoku, R. (2020). Aging and Alzheimer's disease pathology. *Neuropathology*, *40*(1), 22–29. https://doi.org/10.1111/neup.12626

Seo, E. H., Lee, D. Y., Kim, S. G., Kim, K. W., Kim, D. H., Kim, B. jo, Kim, M.-D., Kim, S. Y., Kim, Y. H., Kim, J.-L., Kim, J. W., Moon, S. W., Park, J. H., Ryu, S.-H., Yoon, J. C., Lee, N. J., Lee, C. U., Jhoo, J. H., Choo, ll H., & Woo, J. I. (2011). Validity of the telephone interview for cognitive status (TICS) and modified TICS (TICSm) for mild cognitive imparment (MCI) and dementia screening. *Archives of Gerontology and Geriatrics*, *52*(1), e26–e30. https://doi.org/10.1016/j.archger.2010.04.008

Seow, Y.-X., Ong, P. K. C., & Huang, D. (2016). Odor-Specific Loss of Smell Sensitivity with Age as Revealed by the Specific Sensitivity Test. *Chemical Senses*, *41*(6), 487–495. https://doi.org/10.1093/chemse/bjw051

Serby, M, Larson, P, & Kalkstein, D. (1991). The nature and course of olfactory deficits in Alzheimer's disease. *American Journal of Psychiatry*, *148*(3), 357–360. https://doi.org/10.1176/ajp.148.3.357

Serot, J.-M., Béné, M.-C., & Faure, G. C. (2003). Choroid plexus, aging of the brain, and Alzheimer's disease. *Frontiers in Bioscience: A Journal and Virtual Library*, *8*, s515-521.

Seubert, J., Freiherr, J., Djordjevic, J., & Lundström, J. N. (2013). Statistical localization of human olfactory cortex. *NeuroImage*, *66*, 333–342. https://doi.org/10.1016/j.neuroimage.2012.10.030

Shen, J., Kassir, M. A., Wu, J., Zhang, Q., Zhou, S., Xuan, S. Y., Li, Q., Ye, Y., & Hu, J. (2013). MR Volumetric Study of Piriform-Cortical Amygdala and Orbitofrontal Cortices: The Aging Effect. *PLoS ONE*, *8*(9), e74526. https://doi.org/10.1371/journal.pone.0074526

Shepherd, G. M. (2006). Smell images and the flavour system in the human brain. *Nature*, *444*(7117), 316–321. https://doi.org/10.1038/nature05405

Shi, F., Liu, B., Zhou, Y., Yu, C., & Jiang, T. (2009). Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: Meta-analyses of MRI studies. *Hippocampus*, *19*(11), 1055–1064. https://doi.org/10.1002/hipo.20573

Shohayeb, B., Diab, M., Ahmed, M., & Ng, D. C. H. (2018). Factors that influence adult neurogenesis as potential therapy. *Translational Neurodegeneration*, *7*(1), 4. https://doi.org/10.1186/s40035-018-0109-9

Shukla, V., Skuntz, S., & Pant, H. C. (2012). Deregulated Cdk5 Activity Is Involved in Inducing Alzheimer's Disease. *Archives of Medical Research*, *43*(8), 655–662. https://doi.org/10.1016/j.arcmed.2012.10.015

Siegel, J. K., Wroblewski, K. E., McClintock, M. K., & Pinto, J. M. (2019). Olfactory dysfunction persists after smoking cessation and signals increased cardiovascular risk. *International Forum of Allergy & Rhinology*, *9*(9), 977–985. https://doi.org/10.1002/alr.22357

Šimić, G., Babić Leko, M., Wray, S., Harrington, C., Delalle, I., Jovanov-Milošević, N., Bažadona, D., Buée, L., de Silva, R., Di Giovanni, G., Wischik, C., & Hof, P. (2016). Tau Protein Hyperphosphorylation and Aggregation in Alzheimer's Disease and Other Tauopathies, and Possible Neuroprotective Strategies. *Biomolecules*, *6*(1), 6. https://doi.org/10.3390/biom6010006

Simpson, P. J., Miller, I., Moon, C., Hanlon, A. L., Liebl, D. J., & Ronnett, G. V. (2002). Atrial Natriuretic Peptide Type C Induces a Cell-Cycle Switch from Proliferation to Differentiation in Brain-Derived Neurotrophic Factor- or Nerve Growth Factor-Primed Olfactory Receptor Neurons. *The Journal of Neuroscience*, *22*(13), 5536–5551. https://doi.org/10.1523/JNEUROSCI.22-13-05536.2002

Sinding, C., Puschmann, L., & Hummel, T. (2014). Is the Age-Related Loss in Olfactory Sensitivity Similar for Light and Heavy Molecules? *Chemical Senses*, *39*(5), 383–390. https://doi.org/10.1093/chemse/bju004

Skup, M., Zhu, H., Wang, Y., Giovanello, K. S., Lin, J., Shen, D., Shi, F., Gao, W., Lin, W., Fan, Y., & Zhang, H. (2011). Sex differences in grey matter atrophy patterns among AD and aMCI patients: Results from ADNI. *NeuroImage*, *56*(3), 890–906. https://doi.org/10.1016/j.neuroimage.2011.02.060

Small, D. H., & Cappai, R. (2006). Alois Alzheimer and Alzheimer's disease: A centennial perspective. *Journal of Neurochemistry*, *99*(3), 708–710. https://doi.org/10.1111/j.1471-4159.2006.04212.x

Smith, S. M., Miller, K. L., Salimi-Khorshidi, G., Webster, M., Beckmann, C. F., Nichols, T. E., Ramsey, J. D., & Woolrich, M. W. (2011). Network modelling methods for FMRI. *NeuroImage*, *54*(2), 875–891. https://doi.org/10.1016/j.neuroimage.2010.08.063

Smitka, M., Puschmann, S., Buschhueter, D., Gerber, J. C., Witt, M., Honeycutt, N., Abolmaali, N., & Hummel, T. (2012). Is there a correlation between hippocampus and amygdala volume and olfactory function in healthy subjects? *NeuroImage*, *59*(2), 1052–1057. https://doi.org/10.1016/j.neuroimage.2011.09.024

Smyth, K. A., Fritsch, T., Cook, T. B., McClendon, M. J., Santillan, C. E., & Friedland, R. P. (2004). Worker functions and traits associated with occupations and the development of AD. *Neurology*, *63*(3), 498–503. https://doi.org/10.1212/01.WNL.0000133007.87028.09

Snowden, S. G., Ebshiana, A. A., Hye, A., Pletnikova, O., O'Brien, R., Yang, A., Troncoso, J., Legido-Quigley, C., & Thambisetty, M. (2019). Neurotransmitter Imbalance in the Brain and Alzheimer's Disease Pathology. *Journal of Alzheimer's Disease*, *72*(1), 35–43. https://doi.org/10.3233/JAD-190577

Snowdon, D. A. (1997). Aging and Alzheimer's Disease: Lessons From the Nun Study. *The Gerontologist*, *37*(2), 150–156. https://doi.org/10.1093/geront/37.2.150

Sohrabi, H. R., Bates, K. A., Weinborn, M. G., Johnston, A. N. B., Bahramian, A., Taddei, K., Laws, S. M., Rodrigues, M., Morici, M., Howard, M., Martins, G., Mackay-Sim, A., Gandy, S. E., & Martins, R. N. (2012a). Olfactory discrimination predicts cognitive decline among community-dwelling older adults. *Translational Psychiatry*, *2*(5), e118–e118. https://doi.org/10.1038/tp.2012.43

Sohrabi, H. R., Bates, K. A., Weinborn, M. G., Johnston, A. N. B., Bahramian, A., Taddei, K., Laws, S. M., Rodrigues, M., Morici, M., Howard, M., Martins, G., Mackay-Sim, A., Gandy, S. E., & Martins, R. N. (2012b). Olfactory discrimination predicts cognitive decline among community-dwelling older adults. *Translational Psychiatry*, *2*(5), e118–e118. https://doi.org/10.1038/tp.2012.43

Solfrizzi, V., Panza, F., Frisardi, V., Seripa, D., Logroscino, G., Imbimbo, B. P., & Pilotto, A. (2011). Diet and Alzheimer's disease risk factors or prevention: The current evidence. *Expert Review of Neurotherapeutics*, *11*(5), 677–708. https://doi.org/10.1586/ern.11.56

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., Iwatsubo, T., Jack, C. R., Kaye, J., Montine, T. J., Park, D. C., Reiman, E. M., Rowe, C. C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M. C., Thies, B., Morrison-Bogorad, M., … Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, *7*(3), 280–292. https://doi.org/10.1016/j.jalz.2011.03.003

Stadlbauer, A., Kaltenhäuser, M., Buchfelder, M., Brandner, S., Neuhuber, W. L., & Renner, B. (2016). Spatiotemporal Pattern of Human Cortical and Subcortical Activity during Early-Stage Odor Processing. *Chemical Senses*, *41*(9), 783–794. https://doi.org/10.1093/chemse/bjw074

Staff, R. T., Murray, A. D., Deary, I. J., & Whalley, L. J. (2004). What provides cerebral reserve? *Brain*, *127*(5), 1191–1199. https://doi.org/10.1093/brain/awh144

Stamler, J. (2000). Relationship of Baseline Serum Cholesterol Levels in 3 Large Cohorts of Younger Men to Long-term Coronary, Cardiovascular, and All-Cause Mortality and to Longevity. *JAMA*, *284*(3), 311. https://doi.org/10.1001/jama.284.3.311

Stamps, J. J., Bartoshuk, L. M., & Heilman, K. M. (2013). A brief olfactory test for Alzheimer's disease. *Journal of the Neurological Sciences*, *333*(1–2), 19–24. https://doi.org/10.1016/j.jns.2013.06.033

Stanford, P. M., Shepherd, C. E., Halliday, G. M., Brooks, W. S., Schofield, P. W., Brodaty, H., Martins, R. N., Kwok, J. B. J., & Schofield, P. R. (2003). Mutations in the tau gene that cause an increase in three repeat tau and frontotemporal dementia. *Brain: A Journal of Neurology*, *126*(Pt 4), 814–826.

Stelzmann, R. A., Norman Schnitzlein, H., & Reed Murtagh, F. (1995). Alzheimer's 1907 paper, "ber eine eigenartige erkankung der hirnrinde." *Clinical Anatomy*, *8*(6), 429–431. https://doi.org/10.1002/ca.980080612

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *Journal of the International Neuropsychological Society: JINS*, *8*(3), 448–460.

Stern, Y. (2006). Cognitive reserve and Alzheimer disease. *Alzheimer Disease and Associated Disorders*, *20*(2), 112–117. https://doi.org/10.1097/01.wad.0000213815.20177.19

Stoub, T. R., Bulgakova, M., Leurgans, S., Bennett, D. A., Fleischman, D., Turner, D. A., & deToledo-Morrell, L. (2005). MRI predictors of risk of incident Alzheimer disease: A longitudinal study. *Neurology*, *64*(9), 1520–1524. https://doi.org/10.1212/01.WNL.0000160089.43264.1A

Struble, R. G., & Clark, H. B. (1992). Olfactory bulb lesions in alzheimer's disease. *Neurobiology of Aging*, *13*(4), 469–473. https://doi.org/10.1016/0197-4580(92)90074-8

Sundermann, E. E., Gilbert, P. E., & Murphy, C. (2007). Apolipoprotein E ε4 Genotype and Gender: Effects on Memory. *The American Journal of Geriatric Psychiatry*, *15*(10), 869–878. https://doi.org/10.1097/JGP.0b013e318065415f

Sundermann, E. E., Rubin, L. H., Lipton, R. B., Landau, S., & Maki, P. M. (2017). Does the Female Advantage in Verbal Memory Contribute to Underestimating Alzheimer's Disease Pathology in Women versus Men? *Journal of Alzheimer's Disease*, *56*(3), 947–957. https://doi.org/10.3233/JAD-160716

Suzuki, Y., Critchley, H. D., Suckling, J., Fukuda, R., Williams, S. C. R., Andrew, C., Howard, R., Ouldred, E., Bryant, C., Swift, C. G., & Jackson, S. H. D. (2001). Functional Magnetic Resonance Imaging of Odor Identification: The Effect of Aging. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*(12), M756–M760. https://doi.org/10.1093/gerona/56.12.M756

Tamnes, C. K., Walhovd, K. B., Dale, A. M., Østby, Y., Grydeland, H., Richardson, G., Westlye, L. T., Roddey, J. C., Hagler, D. J., Due-Tønnessen, P., Holland, D., & Fjell, A. M. (2013). Brain development and aging: Overlapping and unique patterns of change. *NeuroImage*, *68*, 63–74. https://doi.org/10.1016/j.neuroimage.2012.11.039

Temmel, A. F. P., Quint, C., Schickinger-Fischer, B., Klimek, L., Stoller, E., & Hummel, T. (2002). Characteristics of olfactory disorders in relation to major causes of olfactory loss. *Archives of Otolaryngology--Head & Neck Surgery*, *128*(6), 635–641. https://doi.org/10.1001/archotol.128.6.635

Terribilli, D., Schaufelberger, M. S., Duran, F. L. S., Zanetti, M. V., Curiati, P. K., Menezes, P. R., Scazufca, M., Amaro, E., Leite, C. C., & Busatto, G. F. (2011). Age-related gray matter volume changes in the brain during non-elderly adulthood. *Neurobiology of Aging*, *32*(2), 354–368. https://doi.org/10.1016/j.neurobiolaging.2009.02.008

Thiermann, F., & Buchbauer, G. (2017). Influence of drugs on smelling capacity. A review. *Flavour and Fragrance Journal*, *32*(3), 147–158. https://doi.org/10.1002/ffj.3368

Thomann, P. A., Dos Santos, V., Seidl, U., Toro, P., Essig, M., & Schröder, J. (2009). MRI-Derived Atrophy of the Olfactory Bulb and Tract in Mild Cognitive Impairment and Alzheimer's Disease. *Journal of Alzheimer's Disease*, *17*(1), 213–221. https://doi.org/10.3233/JAD-2009-1036

Thomann, P. A., Dos Santos, V., Toro, P., Schönknecht, P., Essig, M., & Schröder, J. (2009). Reduced olfactory bulb and tract volume in early Alzheimer's disease—A MRI study. *Neurobiology of Aging*, *30*(5), 838–841. https://doi.org/10.1016/j.neurobiolaging.2007.08.001

Thompson, P. M., Hayashi, K. M., de Zubicaray, G., Janke, A. L., Rose, S. E., Semple, J., Herman, D., Hong, M. S., Dittmer, S. S., Doddrell, D. M., & Toga, A. W. (2003). Dynamics of gray matter loss in

Alzheimer's disease. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(3), 994–1005.

Thompson, P. M., Hayashi, K. M., Dutton, R. A., Chiang, M.-C., Leow, A. D., Sowell, E. R., De Zubicaray, G., Becker, J. T., Lopez, O. L., Aizenstein, H. J., & Toga, A. W. (2007). Tracking Alzheimer's Disease. *Annals of the New York Academy of Sciences*, *1097*(1), 183–214. https://doi.org/10.1196/annals.1379.017

Tobin, M. K., Musaraca, K., Disouky, A., Shetti, A., Bheri, A., Honer, W. G., Kim, N., Dawe, R. J., Bennett, D. A., Arfanakis, K., & Lazarov, O. (2019). Human Hippocampal Neurogenesis Persists in Aged Adults and Alzheimer's Disease Patients. *Cell Stem Cell*, *24*(6), 974-982.e3. https://doi.org/10.1016/j.stem.2019.05.003

Toga, A. W., & Thompson, P. M. (2003). Mapping brain asymmetry. *Nature Reviews Neuroscience*, *4*(1), 37–48. https://doi.org/10.1038/nrn1009

Traschütz, A., Enkirch, S. J., Polomac, N., Widmann, C. N., Schild, H. H., Heneka, M. T., & Hattingen, E. (2020). The Entorhinal Cortex Atrophy Score Is Diagnostic and Prognostic in Mild Cognitive Impairment. *Journal of Alzheimer's Disease*, *75*(1), 99–108. https://doi.org/10.3233/JAD-181150

Tremblay, C., Mei, J., & Frasnelli, J. (2020). Olfactory bulb surroundings can help to distinguish Parkinson's disease from non-parkinsonian olfactory dysfunction. *NeuroImage: Clinical*, *28*, 102457. https://doi.org/10.1016/j.nicl.2020.102457

Trollor, J. N., & Valenzuela, M. J. (2001). Brain Ageing in the New Millennium. *Australian & New Zealand Journal of Psychiatry*, *35*(6), 788–805. https://doi.org/10.1046/j.1440-1614.2001.00969.x

Tunnard, C., Whitehead, D., Hurt, C., Wahlund, Lo., Mecocci, P., Tsolaki, M., Vellas, B., Spenger, C., Kłoszewska, I., Soininen, H., Lovestone, S., Simmons, A., & on behalf of the AddNeuroMed

Consortium. (2011). Apathy and cortical atrophy in Alzheimer's disease. *International Journal of Geriatric Psychiatry*, *26*(7), 741–748. https://doi.org/10.1002/gps.2603

Turetsky, B. I. (2000). Reduced Olfactory Bulb Volume in Patients With Schizophrenia. *American Journal of Psychiatry*, *157*(5), 828–830. https://doi.org/10.1176/appi.ajp.157.5.828

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., & Joliot, M. (2002). Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. *NeuroImage*, *15*(1), 273–289. https://doi.org/10.1006/nimg.2001.0978

Ugurlu, B. N., Akdogan, O., Yilmaz, Y. A., Yapar, D., Aktar Ugurlu, G., Yerlikaya, H. S., & Aslan Felek, S. (2021). Quantitative evaluation and progress of olfactory dysfunction in COVID-19. *European Archives of Oto-Rhino-Laryngology*, *278*(7), 2363–2369. https://doi.org/10.1007/s00405-020-06516-4

Uysal, G., & Ozturk, M. (2020). Hippocampal atrophy based Alzheimer's disease diagnosis via machine learning methods. *Journal of Neuroscience Methods*, *337*, 108669. https://doi.org/10.1016/j.jneumeth.2020.108669

Valenzuela, M. J., Matthews, F. E., Brayne, C., Ince, P., Halliday, G., Kril, J. J., Dalton, M. A., Richardson, K., Forster, G., & Sachdev, P. S. (2012). Multiple Biological Pathways Link Cognitive Lifestyle to Protection from Dementia. *Biological Psychiatry*, *71*(9), 783–791. https://doi.org/10.1016/j.biopsych.2011.07.036

Van Erum, J., Van Dam, D., & De Deyn, P. P. (2019). Alzheimer's disease: Neurotransmitters of the sleep-wake cycle. *Neuroscience & Biobehavioral Reviews*, *105*, 72–80. https://doi.org/10.1016/j.neubiorev.2019.07.019

Vasavada, M. M., Wang, J., Eslinger, P. J., Gill, D. J., Sun, X., Karunanayaka, P., & Yang, Q. X. (2015). Olfactory Cortex Degeneration in Alzheimer's Disease and Mild Cognitive Impairment. *Journal of Alzheimer's Disease*, *45*(3), 947–958. https://doi.org/10.3233/JAD-141947

Velayudhan, L. (2015). Smell identification function and Alzheimer's disease: A selective review. *Current Opinion in Psychiatry*, *28*(2), 173–179. https://doi.org/10.1097/YCO.0000000000000146

Vermeulen, J. M., Schirmbeck, F., Blankers, M., van Tricht, M., Bruggeman, R., van den Brink, W., de Haan, L., Genetic Risk and Outcome of Psychosis (GROUP) investigators, van Amelsvoort, T., Alizadeh, B. Z., Bartels-Velthuis, A. A., van Beveren, N. J., Bruggeman, R., Cahn, W., de Haan, L., Delespaul, P., Meijer, C. J., Myin-Germeys, I., Kahn, R. S., … van Winkel, R. (2018). Association Between Smoking Behavior and Cognitive Functioning in Patients With Psychosis, Siblings, and Healthy Control Subjects: Results From a Prospective 6-Year Follow-Up Study. *American Journal of Psychiatry*, *175*(11), 1121–1128. https://doi.org/10.1176/appi.ajp.2018.18010069

Voss. (2010). Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Frontiers in Aging Neuroscience*. https://doi.org/10.3389/fnagi.2010.00032

Wang, J., Eslinger, P. J., Doty, R. L., Zimmerman, E. K., Grunfeld, R., Sun, X., Meadowcroft, M. D., Connor, J. R., Price, J. L., Smith, M. B., & Yang, Q. X. (2010). Olfactory deficit detected by fMRI in early Alzheimer's disease. *Brain Research*, *1357*, 184–194. https://doi.org/10.1016/j.brainres.2010.08.018

Wang, J., Eslinger, P. J., Smith, M. B., & Yang, Q. X. (2005). Functional Magnetic Resonance Imaging Study of Human Olfaction and Normal Aging. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*(4), 510–514. https://doi.org/10.1093/gerona/60.4.510

Wang, J., Sun, X., & Yang, Q. X. (2016). Early Aging Effect on the Function of the Human Central Olfactory System. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, glw104. https://doi.org/10.1093/gerona/glw104

Wang, J., Yu, J.-T., Wang, H.-F., Meng, X.-F., Wang, C., Tan, C.-C., & Tan, L. (2015). Pharmacological treatment of neuropsychiatric symptoms in Alzheimer's disease: A systematic review and meta-analysis. *Journal of Neurology, Neurosurgery & Psychiatry*, *86*(1), 101–109. https://doi.org/10.1136/jnnp-2014-308112

Warner, M. A., Youn, T. S., Davis, T., Chandra, A., Marquez de la Plata, C., Moore, C., Harper, C., Madden, C. J., Spence, J., McColl, R., Devous, M., King, R. D., & Diaz-Arrastia, R. (2010). Regionally Selective Atrophy After Traumatic Axonal Injury. *Archives of Neurology*, *67*(11). https://doi.org/10.1001/archneurol.2010.149

Wehling, E., Lundervold, A. J., Espeset, T., Reinvang, I., Bramerson, A., & Nordin, S. (2015). Even cognitively well-functioning adults are unaware of their olfactory dysfunction: Implications for ENT clinicians and researchers. *Rhinology*, *53*(1), 89–94. https://doi.org/10.4193/Rhino14.081

Wehling, E., Naess, H., Wollschlaeger, D., Hofstad, H., Hofstad, H., Bramerson, A., Bende, M., & Nordin, S. (2015). Olfactory dysfunction in chronic stroke patients. *BMC Neurology*, *15*, 199. https://doi.org/10.1186/s12883-015-0463-5

Wehling, E., Nordin, S., Espeseth, T., Reinvang, I., & Lundervold, A. J. (2011). Unawareness of olfactory dysfunction and its association with cognitive functioning in middle aged and old adults. *Archives of Clinical Neuropsychology: The Official Journal of the National Academy of Neuropsychologists*, *26*(3), 260–269. https://doi.org/10.1093/arclin/acr019

Wesson, D. W., Levy, E., Nixon, R. A., & Wilson, D. A. (2010). Olfactory Dysfunction Correlates with Amyloid- Burden in an Alzheimer's Disease Mouse Model. *Journal of Neuroscience*, *30*(2), 505–514. https://doi.org/10.1523/JNEUROSCI.4622-09.2010

Westervelt, H. J., Bruce, J. M., Coon, W. G., & Tremont, G. (2008). Odor identification in mild cognitive impairment subtypes. *Journal of Clinical and Experimental Neuropsychology*, *30*(2), 151–156. https://doi.org/10.1080/13803390701287408

White, T. L., & Kurtz, D. B. (2003). The relationship between metacognitive awareness of olfactory ability and age in people reporting chemosensory disturbances. *The American Journal of Psychology*, *116*(1), 99–110.

Wilcock, G. K. (1983). The Temporal Lobe in Dementia of Alzheimer's Type. *Gerontology*, *29*(5), 320–324. https://doi.org/10.1159/000213133

Wilson, D. A. (2009). Olfactory Cortex Physiology. In *Encyclopedia of Neuroscience* (pp. 95–100). Elsevier. https://doi.org/10.1016/B978-008045046-9.01690-9

Wilson, R. S., Arnold, S. E., Schneider, J. A., Boyle, P. A., Buchman, A. S., & Bennett, D. A. (2009). Olfactory Impairment in Presymptomatic Alzheimer's Disease. *Annals of the New York Academy of Sciences*, *1170*, 730–735. https://doi.org/10.1111/j.1749-6632.2009.04013.x

Wilson, R. S., Arnold, S. E., Schneider, J. A., Tang, Y., & Bennett, D. A. (2007). The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *Journal of Neurology, Neurosurgery & Psychiatry*, *78*(1), 30–35. https://doi.org/10.1136/jnnp.2006.099721

Wilson, R. S., Schneider, J. A., Arnold, S. E., Tang, Y., Boyle, P. A., & Bennett, D. A. (2007). Olfactory Identification and Incidence of Mild Cognitive Impairment in Older Age. *Archives of General Psychiatry*, *64*(7), 802. https://doi.org/10.1001/archpsyc.64.7.802

Wilson, R. S., Yu, L., & Bennett, D. A. (2011). Odor Identification and Mortality in Old Age. *Chemical Senses*, *36*(1), 63–67. https://doi.org/10.1093/chemse/bjq098

Wischik, C. M., Storey, J. M. D., Wischik, D. J., & Harrington, C. R. (2016). Inhibition of Tau Aggregation as a Basis for Treatment and Prevention of Alzheimer's Disease. In *Developing Therapeutics for Alzheimer's Disease* (pp. 385–436). Elsevier. https://doi.org/10.1016/B978-0-12-802173-6.00015-0

Wittenberg, R., Hu, B., Barraza-Araiza, L., & Rehill, A. (2019). *Care Policy and Evaluation Centre, London School of Economics and Political Science* (p. 79). https://www.alzheimers.org.uk/sites/default/files/2019-11/cpec_report_november_2019.pdf

Wittenberg, R., Hu, B., Jagger, C., Kingston, A., Knapp, M., Comas-Herrera, A., King, D., Rehill, A., & Banerjee, S. (2020). Projections of care for older people with dementia in England: 2015 to 2040. *Age and Ageing*, *49*(2), 264–269. https://doi.org/10.1093/ageing/afz154

W.J.P. Henneman, J. D. Sluimer, J. Barnes, W. M. van der Flier, I. C. Sluimer, N. C. Fox, P. Scheltens, H. Vrenken, & F. Barkhof. (2009). Hippocampal atrophy rates in Alzheimer disease: Added value over whole brain volume measures. *Neurology*, *72*(11), 999–1007. https://doi.org/10.1212/01.wnl.0000344568.09360.31

Woo, C.-W., Krishnan, A., & Wager, T. D. (2014). Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. *NeuroImage*, *91*, 412–419. https://doi.org/10.1016/j.neuroimage.2013.12.058

Woods, B., Aguirre, E., Spector, A. E., & Orrell, M. (2012). Cognitive stimulation to improve cognitive functioning in people with dementia. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD005562.pub2

Wysocki, C. J., Dorries, K. M., & Beauchamp, G. K. (1989). Ability to perceive androstenone can be acquired by ostensibly anosmic people. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(20), 7976–7978. https://doi.org/10.1073/pnas.86.20.7976

Wyss-Coray, T. (2016). Ageing, neurodegeneration and brain rejuvenation. *Nature*, *539*(7628), 180–186. https://doi.org/10.1038/nature20411

Wyss-Coray, T., & Rogers, J. (2012). Inflammation in Alzheimer Disease—A Brief Review of the Basic Science and Clinical Literature. *Cold Spring Harbor Perspectives in Medicine*, *2*(1), a006346–a006346. https://doi.org/10.1101/cshperspect.a006346

Xia, D., Watanabe, H., Wu, B., Lee, S. H., Li, Y., Tsvetkov, E., Bolshakov, V. Y., Shen, J., & Kelleher, R. J. (2015). Presenilin-1 Knockin Mice Reveal Loss-of-Function Mechanism for Familial Alzheimer's Disease. *Neuron*, *85*(5), 967–981. https://doi.org/10.1016/j.neuron.2015.02.010

Xia, X., Jiang, Q., McDermott, J., & Han, J.-D. J. (2018). Aging and Alzheimer's disease: Comparison and associations from molecular to system level. *Aging Cell*, *17*(5), e12802. https://doi.org/10.1111/acel.12802

Xifra-Porxas, A., Ghosh, A., Mitsis, G. D., & Boudrias, M.-H. (2021). Estimating brain age from structural MRI and MEG data: Insights from dimensionality reduction techniques. *NeuroImage*, *231*, 117822. https://doi.org/10.1016/j.neuroimage.2021.117822

Yao, L., Pinto, J. M., Yi, X., Li, L., Peng, P., & Wei, Y. (2014). Gray matter volume reduction of olfactory cortices in patients with idiopathic olfactory loss. *Chemical Senses*, *39*(9), 755–760. https://doi.org/10.1093/chemse/bju047

Yilmaz, A., Geddes, T., Han, B., Bahado-Singh, R. O., Wilson, G. D., Imam, K., Maddens, M., & Graham, S. F. (2017). Diagnostic Biomarkers of Alzheimer's Disease as Identified in Saliva using 1H NMR-

Based Metabolomics. *Journal of Alzheimer's Disease*, *58*(2), 355–359. https://doi.org/10.3233/JAD-161226

Yousem, D. M., Maldjian, J. A., Hummel, T., Alsop, D. C., Geckle, R. J., Kraut, M. A., & Doty, R. L. (1999). The effect of age on odor-stimulated functional MR imaging. *AJNR. American Journal of Neuroradiology*, *20*(4), 600–608.

Yousem, D. M., Maldjian, J. A., Siddiqi, F., Hummel, T., Alsop, D. C., Geckle, R. J., Bilker, W. B., & Doty, R. L. (1999). Gender effects on odor-stimulated functional magnetic resonance imaging. *Brain Research*, *818*(2), 480–487. https://doi.org/10.1016/S0006-8993(98)01276-1

Yu, H.-L., Chen, Z.-J., Zhao, J.-W., Duan, S.-R., & Zhao, J.-K. (2019). Olfactory Impairment and Hippocampal Volume in a Chinese MCI Clinical Sample. *Alzheimer Disease and Associated Disorders*, *33*(2), 124–128. https://doi.org/10.1097/WAD.0000000000000305

Zador, A., & Mombaerts, P. (2007). Neuronal circuitry and population activity. *Current Opinion in Neurobiology*, *17*(4), 395–396. https://doi.org/10.1016/j.conb.2007.09.001

Zhang, C., & Wang, X. (2017). Initiation of the age-related decline of odor identification in humans: A meta-analysis. *Ageing Research Reviews*, *40*, 45–50. https://doi.org/10.1016/j.arr.2017.08.004

Zhang, J., & Jiao, J. (2015). Molecular Biomarkers for Embryonic and Adult Neural Stem Cell and Neurogenesis. *BioMed Research International*, *2015*, 727542. https://doi.org/10.1155/2015/727542

Zhang, Z., Rowan, N. R., Pinto, J. M., London, N. R., Lane, A. P., Biswal, S., & Ramanathan, M. (2021). Exposure to Particulate Matter Air Pollution and Anosmia. *JAMA Network Open*, *4*(5), e2111606. https://doi.org/10.1001/jamanetworkopen.2021.11606

Zhao, W., Wang, X., Yin, C., He, M., Li, S., & Han, Y. (2019). Trajectories of the Hippocampal Subfields Atrophy in the Alzheimer's Disease: A Structural Imaging Study. *Frontiers in Neuroinformatics*, *13*, 13. https://doi.org/10.3389/fninf.2019.00013

Zou, Y., Lu, D., Liu, L.-P., Zhang, H., & Zhou, Y. (2016). Olfactory dysfunction in Alzheimer's disease. *Neuropsychiatric Disease and Treatment*, 869. https://doi.org/10.2147/NDT.S104886

Zucco, G. M., & Bollini, F. (2011). Odour recognition memory and odour identification in patients with mild and severe major depressive disorders. *Psychiatry Research*, *190*(2–3), 217–220. https://doi.org/10.1016/j.psychres.2011.08.025

Appendix A. Original research article based on chapter 4, experiment 1

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RESEARCH ARTICLE

Unawareness of Olfactory Dysfunction in Older Adults

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Abstract

Deterioration of olfaction is a common phenomenon observed in the senior population. A number of factors may cause this deficit including infections, aging and neurodegenerative diseases. The aim of this study was to evaluate the reliability of the self-report as a measure of olfactory function in seniors. A total of 93 community-dwelling participants (43 men and 50 women) from the Quebec NuAge cohort on Nutrition and Successful Aging participated in the Olfactory Response and Cognition in Aging (ORCA) study. The age range was 80-95 years and all subjects had a telephone mini mental state examination (t-MMSE) score > 18. Individuals were interviewed using a self-report ("do you suffer from smell problems?") and quantitative (University of Pennsylvania Smelling Identification Test (UPSIT)) olfactory tests. Based on the self-report, 81% of the participants claimed to have a normal sense of olfaction. However, based on the UPSIT, 95% of them showed different forms of microsmia. These results reveal that most senior citizens are unaware of their olfactory dysfunction and indicate that an self-report questionnaire is not a valid instrument to assess olfactory function in the aging population.

Keywords

Aging, elderly, Metacognition, Olfaction, Self-evaluation

Abbreviations

NuAge: Quebec NuAge Cohort on Nutrition and Successful Aging; ORCA: Olfactory Response and Cognition in Aging Study; t-MMSE: Telephone Mini Mental State Examination; UPSIT: University of Pennsylvania Smelling Identification Test; AD: Alzheimer Disease; HD: Huntington Disease; MCI: Mild Cognitive Impairement

Introduction

From a phylogenetic perspective, olfaction is deemed one of the oldest sensory systems in mammals [1]. Often taken for granted, the sense of smell is of crucial importance. Although olfaction may appear to have less importance in identifying objects or people compared to vision, this sense plays a crucial social and emotional role which affects an individual's tastes and food preferences daily [2]. Furthermore, olfaction represents a strong asset when it comes to detecting danger through odours such as gas leaks and/or other toxic fumes, smoke and rotting food. Strong evidence indicates that olfactory dysfunction occurs with aging [3]. From this perspective, olfactory dysfunction is a major public health concern and measures need to be put in place to alleviate these dangers.

Several factors have been identified that may cause olfactory dysfunction including stroke, viral infections and aging [4-6]. Age-related olfactory dysfunction is characterized by a significant decrease in adrenergic innervation density in the lamina propria of the olfactory mucosa [7]. Other factors implicated in the loss of olfactory function include air-flow and mucous composition, structure of the olfactory neuroepithelium and bulb, and olfactory processing in the brain [8]. The deterioration of smell due to age is often gradual and therefore not noticeable by the affected individual. Many older subjects with microsmia are completely unaware of

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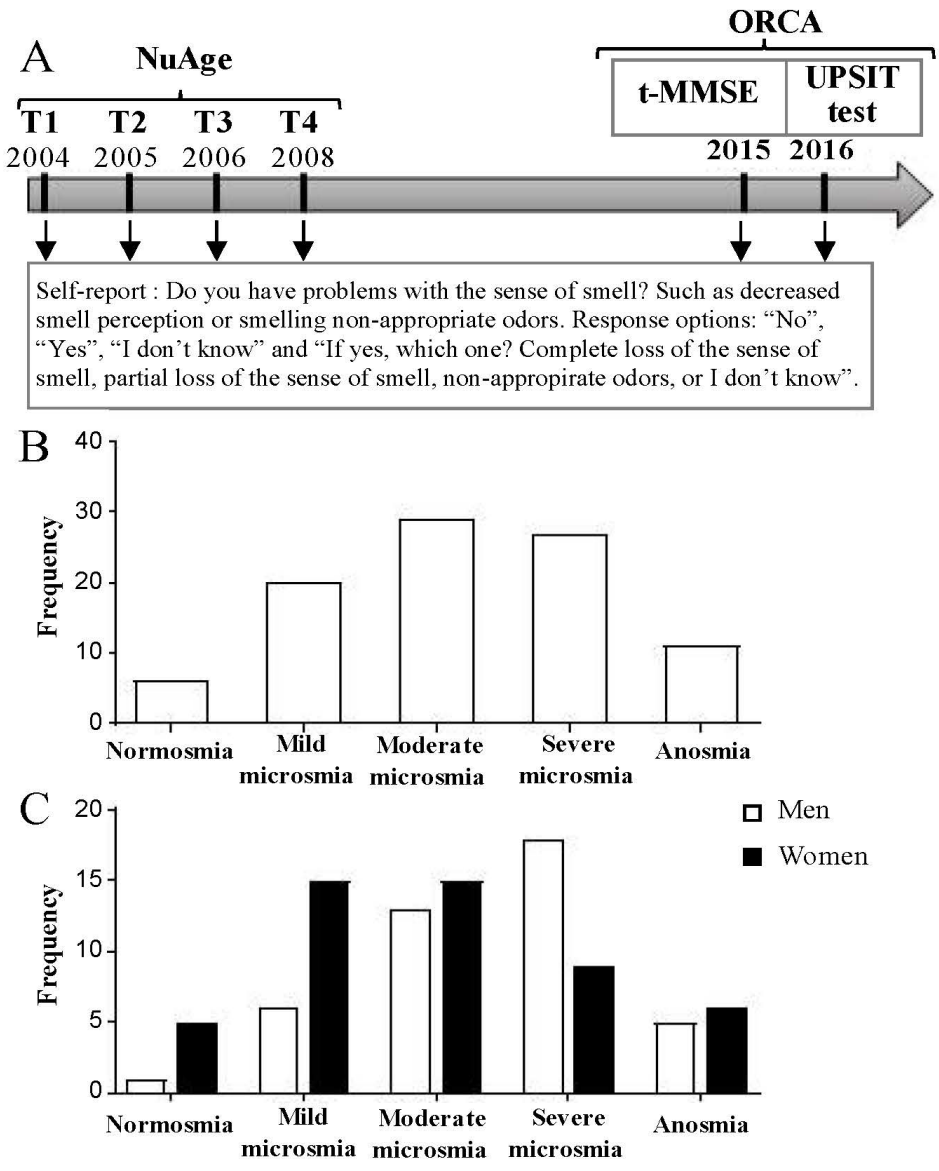

Figure 1: Experimental design of the ORCA study. The self-report was completed at all the time-points (NuAge study: T1- T4, ORCA: 2015 and 2016). A) The last self-report (2016), was done at the same time as the UPSIT test in front of the evaluator. The t-MMSE was done in 2015. Olfactory dysfunction is common among seniors citizen. B) 94% (87/93) of participants have one of the forms of microsmia (males and females combined). C) No difference in the distribution of olfactory status is observed between sex (Chi-squared, p = 0.074).

their olfactory state [9-11]. Unawareness of olfactory impairment may be caused by the lack of attention towards a stimulus. It has been shown that attention is necessary for olfactory consciousness [12] and that the auto-evaluation of the olfactory functions correlates with the quantitative olfactory test scores only after assessing the olfactory function [13]. In addition, many people suffering from olfactory dysfunction tend to mistake their impairment for a taste loss rather than a smell loss [14].

A study performed in Sweden and Norway, which included healthy participants between the age of 45-79, has demonstrated that unawareness of anosmia (complete loss of olfaction) is frequent in older adults (79%) who have healthy cognitive abilities based on a comprehensive neurological assessment [15]. In a similar study, conducted in the United States on adults aged 53 to 79 years, only 20% of subjects who suffered from anosmia were aware of having problems with olfaction [16]. The general consensus in the literature, based on the limited studies conducted thus far, supports the hypothesis that seniors are unaware of their olfactory impairment [10,13,17]. However, there are some studies which demonstrate that elderly subjects were able to estimate their olfactory function [18,19].

Substantial evidence in the literature has shown that olfactory dysfunction is observed in several neurodegenerative diseases, such as Alzheimer disease (AD), Huntington disease (HD) and Stroke and Parkinson disease (PD) among others [20]. Importantly, several studies suggest that olfactory dysfunction may represent an early predictor of future cognitive impairment [21-23]. Indeed, olfactory impairment is currently seen as one of the top predictors of impending PD and is observed in Mild Cognitive Impairment (MCI), a potential precursor of AD [24,25]. This highlights the importance of establishing robust tests that reveal a smell disorder in an older individual.

Importantly, unawareness of olfactory problems may lead to malnutrition and depression [26,27]. In addition, affected individuals will not be able to access their olfactory memories which may contribute to the depression [28]. This becomes a prominent concern considering that depression in individuals with MCI more than doubles the risk for progression to AD [29]. In this report, we wished to determine if the olfactory self-report is a reliable method to determine olfactory function as there is limited information in the literature. We assessed an aging population in the province of Quebec and compared the performance of the self-evaluation method to the quantitative evaluation of olfaction in this same population.

Materials and Methods

Participants

A total of 93 individuals (50 females (age range 80-95), 43 males (age range 80-93)) from Quebec, agreed to participate in the clinical sub-study *Olfactory Response and Cognition in Aging* (ORCA) (Figure 1, given separately). The participants were recruited from the large database of the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge) in which only healthy and cognitively fit adults (> 67 years of age) were included [30]. The NuAge study recruited 1,793 individuals who were assessed annually between 2004-2008. For the ORCA study, initial calls were made to the previous NuAge participants asking if they would consider to be involved in other studies and/or the ORCA sub-study. For those who agreed to be involved, the telephone Mini Mental State Examination (t-MMSE) was done and only cognitively fit adults (≥ 18) were admitted into the ORCA sub-study. Letters were then sent out which included a self-report olfaction questionnaire (identical to original NuAge self-report) (2015) and a consent form. All the subjects signed the consent form in their native language which was approved by the Research Centre of Aging ethical committee (Quebec REB 2015-477). At the time of the NuAge study, no participants reported diagnosed neurological diseases, such as Parkinson disease, Huntington disease and/or Stroke. The characteristics of the participants are presented in Table 1 (Given separately).

Self-Report

NuAge applicants filled in self-report forms about their olfactory state each year from 2004-2008 (T1-T4) which included the questions: "Do you have problems with the sense of smell? Such as decreased smell perception or smelling non-appropriate odor. Response options: "No", "Yes", "I don't know" and "If yes, which one? Complete loss of the sense of smell, partial loss of the sense of smell, non-appropriate odors, or I don't know". In 2015/2016, 93 of these participants, who now make-up the ORCA sample also completed a self-report questionnaire (identical to original NuAge self-report) at the time of the signature of the consent form. Another self-report was completed in front of an evaluator in 2016, at the same time of the assessment of the University of Pennsylvania smell Identification Test (UPSIT) test.

Olfactory Evaluation

The UPSIT, the gold-standard for quantitative as-

Table 1: Descriptive characteristic of study participants.

	Women	Men	p value
Age	85.8 ± 3.8	85.4 ± 3.9	0.58
UPSIT	27.6 ± 6.8	25.3 ± 5.1	0.07
Education (years)	12.5 ± 4.0	15.7 ± 4.2	0.0004***
Smoking			0.009**
Never	31	15	
Past	19	28	
Current	0	0	

Table 2: Participants distribution of qualitative (2015 self-report) and quantitative (UPSIT) level of olfaction.

OLFACTION DIAGNOSIS	Self-report	Women (n = 50)	Men (N = 43)	Total (N = 93)
	Answer	Age (80-95)	Age (80-93)	age (80-95)
Normosmia (%)	No	6 (3/50)	2.3 (1/43)	4.3 (4/93)
	Yes	4 (2/50)	0 (0/43)	2.2 (2/93)
	I don't know	0 (0/50)	0 (0/43)	0 (0/93)
Mildmicrosmia (%)	No	24 (12/50)	16.2 (7/43)	20.4 (19/93)
	Yes	2 (1/50)	0 (0/43)	1.1 (1/93)
	I don't know	2 (2/50)	0 (0/43)	2.2 (2/93)
Moderatemicrosmia (%)	No	28 (14/50)	23.3 (10/43)	25.8 (24/93)
	Yes	2 (1/50)	0 (0/43)	1.1 (1/93)
	I don't know	0 (0/50)	4.7 (2/43)	2.2 (2/93)
Severemicrosmia (%)	No	12 (6/50)	37.2 (16/43)	23.7 (22/93)
	Yes	4 (2/50)	2.3 (1/43)	3.2 (3/93)
	I don't know	2 (1/50)	2.3 (1/43)	2.2 (2/93)
Anosmia (%)	No	6 (3/50)	7 (3/43)	6.5 (6/93)
	Yes	6 (3/50)	2.3 (1/43)	4.3 (4/93)
	I don't know	0 (0/50)	2.3 (1/43)	1.1 (1/93)
Total (%)		100	100	100

sessment of olfaction, is commercially available as the Smell Identification Test (SIT, Sensonics, Inc.) and is the most widely used quantitative olfactory test [20]. It contains four booklets with a total of 40 different odors. Each smell is micro-encapsulated which are released by scratching a lead pencil. The participant must provide the most appropriate answer between four alternatives forced choices. The test is rated on a maximum score of 40 and each score range corresponds to an olfactory diagnosis (described in the UPSIT manual). The staff who conducted the interviews were trained and familiar with the olfactory tests. A $10 compensation was given to each participant to cover incidentals such as parking.

Statistical Analysis

The results of the qualitative and quantitative olfactory tests were first compiled by the administrator who performed them and rechecked twice by two other administrators. The qualitative results taken in 2015/2016 were compared with those taken between 2004 and 2008 (T1 - T4), and then compared to the quantitative results. We also compared the results obtained in 2015 and 2016 together. All statistical analysis was performed with Graphpad Prism 7 software. Cochran's Q tests were used to compare frequencies of self-report answers in time, and chi-square tests to compare frequencies of self-report and objective assessment between sex. T-test between independent groups (age, education and raw UPSIT) and Chi square (smoking status) were used to compare the characteristic of men vs. women in Table 1 (Given separately). The equality of variances was analysed using the F-test and the variance between man and women were not significantly different for all the variables assessed. The level of significance was set at $p < 0.05$. The measure of the sensitivity (correctly identify those with the disease) and specificity (correctly identify those without the disease) of the self-report was calculated by comparing the self-report results with the olfactory status evaluated by the UPSIT score.

Results

The results of the 2015/2016 quantitative olfactory tests are shown in Table 2 (Given separately). According to the quantitative results harvested in 2015/2016, 94% (87/93) of participants suffered from one of the forms of hyposmia (mild (24%), moderate (29%) or severe microsmia (29%) and total anosmia (12%)) (Table 2, Figure 1A, Figure 1B given separately). Even if we took into the account only the most severe forms of olfactory dysfunction (moderate, severe microsmia and anosmia) that are the most likely to affect the safety and quality of life, the percentage was still quite high (70%, 65/93). We then compared the results across sex. The distribution of olfactory status was not significantly different between the men and women in our population (Figure 1C, Chi-squared $p = 0.074$, given separately). However, the percentage of individuals who suffer from mild microsmia was 18% higher in women, while the percentage of severe microsmia was 24% higher in men.

We next compared the quantitative UPSIT olfactory score with the responses on the self-report questionnaire. In sharp contrast to the quantitative results, the qualitative self-report of 2015/2016 demonstrates that 81% (75/93) of participants claimed not to suffer from any problems with olfaction (Figure 2A, given separately). Within these 75 individuals, 95% (71/75) had some form of microsmia. It is worth mentioning that 91% (10/11) of subjects who claimed to have olfactory dys-

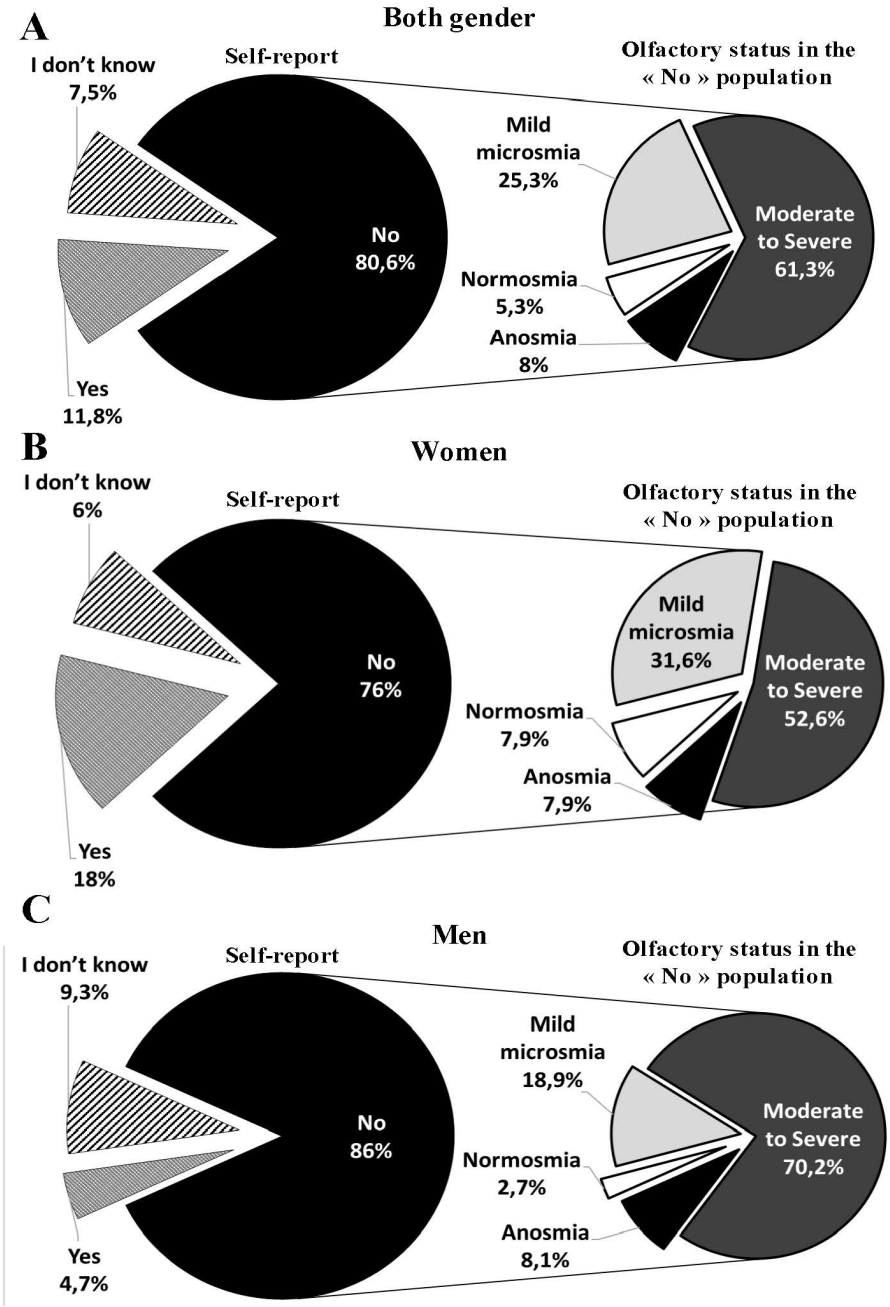

Figure 2: Unawareness of olfactory dysfunction is not sex-related. A) Distribution of the self-report assessment and UPSIT result in the participants (males and females combined) who answered ''No''. B) Distribution of the self-report assessment and UPSIT result in women who answered ''No''. C) Distribution of the self-report assessment and UPSIT result in men who answered ''No''. No significant difference is detected in the olfactory status between men and women for the subjects who answered "No" (Chi-squared, p = 0.11).

function did suffer from it according to UPSIT score. In addition, 7 out of 93 participants reported not knowing their olfactory state and 6 of those individuals had microsmia.

We next looked at the distribution of the different olfaction states in those who claimed not to suffer from olfactory dysfunction by sex (Figure 2B, Figure 2C, given separately). For females, 76% responded that they had no olfactory impairment, however, 92% (35/38) had deficits as detected by the UPSIT (8% (3/38) normosmia, 32% (12/38) microsmia, 37% (14/38) moderate microsmia, 16% (6/38) severe microsmia and 8% (3/38) total anosmia) (Figure 2B, Table 2, Given separately). We found a similar situation in the male population, with 86% declaring no deficits when in fact 97% (36/37) did demonstrate problems with smell according to UPSIT (3% (1/37) normosmia, 19% (7/37) mild microsmia, 27% (10/37) moderate microsmia, 43% (16/37) severe microsmia and 8% (3/37) total anosmia) (Figure 2C, Table 2, given separately). Of note, in the group who claimed not to suffer from any problems with smells, the difference in the frequency of microsomia between men and women was not significant ($p = 0.11$). These results demonstrate that while the majority of individuals in our sample claim not to have any problems with smell, they actually had different forms of microsmia. The overall sensitivity of the self-report was 12.3% and the specificity was 80%.

The evaluation of the individual's assessment of their olfactory function (self-report) was then evaluated over the years 2004 to 2008 (T1-T4), and in 2015/2016 (Figure 3, given separately). Fluctuations in the percentages of individuals who reported "no" to the question "do you suffer from any problems in smell" did not change significantly over the years (Cochran's Q test, $p = 0.11$). The number of individuals reporting no olfactory dysfunction represents the majority of the subjects (81% in 2015). We also compared the results of the self-report done in 2015 and 2016 to see if the presence of an evaluator affected the answer of the participant. Despite the fact that there was no significant variation between the answers in 2015 and 2016 ($p = 0.74$, data not shown), we observed some individual variations. Indeed, 5 of the 11 individuals who reported a problem with smell in 2015 changed the answer to "No" in front of the evaluator. Interestingly, one of these participants reported a complete loss of smell in 2015. Of note, only 4 of the individuals who reported no problem with sense of smell in 2015 changed their answer to yes at 2016 in front of the evaluator.

We then focused on the consistency of the self-report responses by analyzing the proportion of individuals who maintained the same response on their olfactory self-report over time. It may be expected that the elderly participants would change their answer from "no" to "yes" to the question "do you suffer from any problems in smell" as their olfaction deteriorates over the years. However, the opposite effect was noticed. Indeed, 88% of the participant who responded "no" at T1 maintained their answer throughout the 5 timepoints (64/73 participants). In sharp contrast, only 1 out of 8 participants who responded "yes" maintained his answer throughout the years. Despite the fact that almost all the participants who responded "yes" changed their answer over the years, the variation over time was not significant. This may be explained by the small proportion of individuals who claimed to have an olfactory problem in the self-report (Cochran's Q test, "no" $p = 0.001$; "yes" $p = 0.1$).

Discussion

From the quantitative olfactory test (UPSIT), our re-

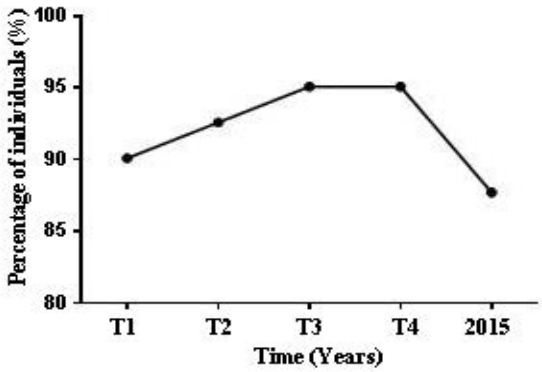

Figure 3: Percentage of self-reporting rates on the state of olfaction of participants did not change between 2004 and 2016. The percentage of individuals who reported no olfactory deficits remained stable over time (Cochran's Q test, $p = 0.11$). For the purpose of the statistical analysis, the answer 'I don't know' were discarded from the analysis.

sults demonstrate that 94% (87/93) of the participants have olfactory problems. In contrast, the result of the self-report (2015) demonstrates that 81% of participants claimed not to suffer from any problems with olfaction. Furthermore, the majority of the participants who appeared to be aware of their olfactory dysfunction in 2004 then subsequently changed their answer about their olfaction as they became unaware of it. Additionally, our results show that unawareness of olfactory dysfunction is not sex-related. The fact that humans undergo a gradual decline in olfaction with age [20] may make them less aware of their loss over time [11,31]. Indeed, the objective of this study was to determine whether unawareness of olfactory impairment is a common problem in cognitively well-functioning seniors. Furthermore, the goal was to evaluate the reliability of the olfactory self-reports as a way to determine olfactory capabilities among the elderly in the clinic. These objectives were carried out by comparing the qualitative test of olfaction with the quantitative test (UPSIT) on participants aged 80 to 95 years. Despite the relatively high specificity (correct identification of normosmia) of the self-report (80%), the sensitivity (correct identification of olfactory dysfunction) was very poor (12%) suggesting that the self-report is not a reliable test to diagnose olfactory impairment among the senior population.

Similar to other studies, our results demonstrate that the majority of elderly individuals have olfactory deficits [16]. However, what is more alarming is that 69% (95% if we include mild microsomia) of the participants who claimed to have no olfactory dysfunction were not aware of their actual olfactory dysfunction. The lack of association between the self-report and measured olfaction may also be a result of the fact that the majority of studies on olfaction in the elderly used a self-report that include only a single question [10,11,15]. There are only four studies on olfactory awareness that use the multiple questions self-report [10,15,18,32]. One of these studies, done on only women, shows an association between the self-report and olfactory function [18], another study demonstrated an association only in the population with Alzheimer's disease and the others show no correlation [32].

Interestingly, it has been shown that older individuals are not more likely in general to make errors in the estimation of their faculties compared to the younger individuals. However, they tend to overestimate their olfactive faculties, while the younger population are more likely to underestimate them (White & Kurtz, 2003). This represents a significant practical danger for seniors as more than 32% women and 16% men over 65 years old was living alone in 2011 according to statistics Canada [33] and who may be exposed to several risks of everyday life such as detecting gas leaks, toxic substances, smoke or outdated food.

It should be noted that from 2004 to 2016, only 10% of the participants switched their answer in the self-report from no to yes. In the opposite case, 45% of participants changed their statement from yes to no, a rather significant change. We also compared the proportion of the subjects who maintained the same answer through the years. 86% of the subjects who declared 'no' maintained their answer (10% changed their answer for 'yes' and 5% for 'I don't know'). However, only 27% of the individuals maintained 'yes' as an answer throughout the years (45% changed their answer to 'No' and 27% for 'I don't know'). This may be explained by the fact that unawareness of olfactory dysfunction is common in older people and is not improving with increasing age. The fact that 72% of the participants have disowned their olfactory problems may be due to a deterioration of their olfactory memory since the majority of this specific subgroup of participants (7/11) seemed to suffer from anosmia or a severe form of microsmia. In other words, even though these participants had previously acknowledged their olfactory impairment, they then seemed to forget that they had microsmia. These results suggest that self-reports are a very subjective measure of olfaction and are not sensitive nor specific enough to reflect the true olfactory state of the individual.

Olfactory dysfunction has a high prevalence in neurodegenerative diseases such as AD, PD and HD [8,34] and strong evidence demonstrates that this is an early event in a number of neurological diseases and may be a harbinger of future cognitive impairment [35,36]. Based on our results and the results of others, it becomes critical to establish quantitative olfactory testing in the clinic for seniors in order to provide education around non-olfactory avoidance of hazardous events (smoke and gas detectors, dating food, fire-escape plans) and to highlight treatment alternatives for olfactory dysfunction [37-40] and mental issues related to olfactory loss (depression, loss of appetite). Importantly, quantitative olfactory testing would enable reliable and robust levels of olfactory functions to be determined in the elderly in order to triage which senior individuals should then go on to have other markers assessed for neurodegenerative diseases. To this day there is no widely adopted policy or clinical algorithm in place for the detection of individuals that have early dementia or indeed may be on the road to cognitive impairment and a neurodegenerative disease. This is despite the fact that there are preventive strategies for dementia-related diseases that have been reliably shown to delay disease progression (healthy eating, exercise and social interactions amongst others) [41-45]. Significant savings of lives, and in health-related expenses, could be realized should these measures be put into wide spread clinical use.

Screening pocket olfactory tests need to become routine in the health-care practices. A number of such tests are available including Smell diskettes which can

be reused. The UPSIT remains to date the most commonly used test for olfaction. However, there are time constraints with this test. There are several shorter olfaction tests that are less expensive, and the data would suggest they may be suitable as a first screen (Supplemental Table 1, Given separately). As an example, The Pocket Smell is a very brief test that contains only three different odors and has been shown to be highly sensitive in detecting olfactory dysfunction [46]. If the individual misidentifies one of the three odors, then the B-SIT and/or the UPSIT (which contains 40 odors) could be used to further investigate the level of microsmia. In order to consider the best interest of seniors, as well as the financial burden of potential impending neurodegenerative diseases, we would suggest doing olfactory screening, using a quantitative olfaction identification test, on individuals > 50 years as part of their annual medical check-up.

In conclusion, the results of this clinical study suggest that unawareness of olfactory dysfunction is very common in the aging population and that the self-report is not a reliable method for assessing olfactory status of seniors. Until this day, some studies consider the reliability of self-reports against quantitative tests still debatable [18,32]. However, the majority of the findings in the literature supports the opposite [10,13,17]. Our study demonstrates that the quantitative tests are more reliable, and more objective, when it comes to evaluating an individual's sense of smell. Our findings conclude that there is discrepancy between the olfactory self-reports and quantitative tests.

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Conflict of Interests

The authors declare that they do not have any conflicts of interest.

Authorship Contribution

AL, MLB, MA, KB and RKG performed the olfactory test on the participants. AL, MA and MLB analyzed the self-reports and the olfactory tests results. AL and MLB performed to statistical analysis. AL, MA, MLB and RKG wrote the manuscript. PG was involved in the original NuAge study.

References

1. Brattoli M, de Gennaro G, de Pinto V, Loiotile AD, Lovascio S, et al. (2011) Odour detection methods: olfactometry and chemical sensors. Sensors 11: 5290-5322.
2. Sarafoleanu C, Mella C, Georgescu M, Perederco C (2009) The importance of the olfactory sense in the human behavior and evolution. J Med Life 2: 196-198.
3. Zhang C, Wang X (2017) Initiation of the age-related decline of odor identification in humans: A meta-analysis. Ageing Research Reviews 40: 45-50.
4. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, et al. (1984) Smell identification ability: changes with age. Science 226: 1441-1443.
5. Lee DY, Lee WH, Wee JH, Kim J-W (2014) Prognosis of postviral olfactory loss: follow-up study for longer than one year. Am J Rhinol Allergy 28: 419-422.
6. Wehling E, Naess H, Wollschlaeger D, Hofstad H, Hofstad H, et al. (2015) Olfactory dysfunction in chronic stroke patients. BMC Neurol 15: 199.
7. Chen Y, Getchell TV, Sparks DL, Getchell ML (1993) Patterns of adrenergic and peptidergic innervation in human olfactory mucosa: age-related trends. The Journal of Comparative Neurology 334: 104-116.
8. Alves J, Petrosyan A, Magalhães R (2014) Olfactory dysfunction in dementia. World J Clin Cases 2: 661-667.
9. Schiffman SS (1993) Perception of taste and smell in elderly persons. Crit Rev Food Sci Nutr 33: 17-26.
10. Wehling E, Nordin S, Espeseth T, Reinvang I, Lundervold AJ (2011) Unawareness of olfactory dysfunction and its association with cognitive functioning in middle aged and old adults. Arch Clin Neuropsychol 26: 260-269.
11. White TL, Kurtz DB (2003) The relationship between metacognitive awareness of olfactory ability and age in people reporting chemosensory disturbances. Am J Psychol 116: 99-110.
12. Keller A (2011) Attention and olfactory consciousness. Frontiers in Psychology 2: 380.
13. Landis BN, Hummel T, Hugentobler M, Giger R, Lacroix JS (2003) Ratings of overall olfactory function. Chemical Senses 28: 691-694.
14. Doty RL, Reyes PF, Gregor T (1987) Presence of both odor identification and detection deficits in Alzheimer's disease. Brain Research Bulletin 18: 597-600.
15. Wehling E, Lundervold AJ, Espeset T, Reinvang I, Bramerson A, et al. (2015) Even cognitively well-functioning adults are unaware of their olfactory dysfunction: Implications for ENT clinicians and researchers. Rhinology 53: 89-94.

16. Murphy C, Schubert CR, Cruickshanks KJ, Klein BEK, Klein R, et al. (2002) Prevalence of olfactory impairment in older adults. JAMA 288: 2307-2312.

17. Nordin S, Monsch AU, Murphy C (1995) Unawareness of smell loss in normal aging and Alzheimer's disease: discrepancy between self-reported and diagnosed smell sensitivity. J Gerontol B Psychol Sci Soc Sci 50: 187-192.

18. Rawal S, Hoffman HJ, Chapo AK, Duffy VB (2014) Sensitivity and Specificity of Self-Reported Olfactory Function in a Home-Based Study of Independent-Living, Healthy Older Women. Chemosens Percept 7: 108-116.

19. Rawal S, Hoffman HJ, Honda M, Huedo-Medin TB, Duffy VB (2015) The Taste and Smell Protocol in the 2011-2014 US National Health and Nutrition Examination Survey (NHANES): Test-Retest Reliability and Validity Testing. Chemosens Percept 8: 138-148.

20. Doty RL (2017) Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? Lancet Neurol 16: 478-488.

21. Barresi M, Ciurleo R, Giacoppo S, Foti Cuzzola V, Celi D, et al. (2012) Evaluation of olfactory dysfunction in neurodegenerative diseases. Journal of the Neurological Sciences 323: 16-24.

22. Devanand DP, Michaels-Marston KS, Liu X, Pelton GH, Padilla M, et al. (2000) Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. The American Journal of Psychiatry 157: 1399-1405.

23. Growdon ME, Schultz AP, Dagley AS, Amariglio RE, Hedden T, et al. (2015) Odor identification and Alzheimer disease biomarkers in clinically normal elderly. Neurology 84: 2153-2160.

24. Picillo M, Pellecchia MT, Erro R, Amboni M, Vitale C, et al. (2014) The use of University of Pennsylvania Smell Identification Test in the diagnosis of Parkinson's disease in Italy. Neurol Sci 35: 379-383.

25. Liepelt-Scarfone I, Gauss K, Maetzler W, Muller K, Bormann C, et al. Evaluation of progression markers in the premotor phase of Parkinson's disease: the progression markers in the premotor phase study. Neuroepidemiology 41: 174-182.

26. Cook A, Pfeiffer LM, Thiele S, Coenen VA, Döbrössy MD (2017) Olfactory discrimination and memory deficits in the Flinders Sensitive Line rodent model of depression. Behavioural processes 143: 25-29.

27. Temmel AFP, Quint C, Schickinger-Fischer B, Klimek L, Stoller E, et al. (2002) Characteristics of olfactory disorders in relation to major causes of olfactory loss. Arch Otolaryngol Head Neck Surg 128: 635-641.

28. Zucco GM, Bollini F (2011) Odour recognition memory and odour identification in patients with mild and severe major depressive disorders. Psychiatry Res 190: 217-220.

29. Boyle PA, Wilson RS, Aggarwal NT, Tang Y, Bennett DA (2006) Mild cognitive impairment: risk of Alzheimer disease and rate of cognitive decline. Neurology 67: 441-445.

30. Gaudreau P, Morais JA, Shatenstein B, Gray-Donald K, Khalil A, et al. (2007) Nutrition as a determinant of successful aging: description of the Quebec longitudinal study Nuage and results from cross-sectional pilot studies. Rejuvenation Res 10: 377-386.

31. Krajnik J, Kollndorfer K, Notter LA, Mueller CA, Schopf V (2015) The impact of olfactory dysfunction on interoceptive awareness. Psychophysiology 52: 263-268.

32. Djordjevic J, Jones-Gotman M, De Sousa K, Chertkow H (2008) Olfaction in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 29: 693-706.

33. Canada S (2012) Living arrangements of seniors. Ottawa, Canada.

34. Duff K, McCaffrey RJ, Solomon GS (2002) The Pocket Smell Test: successfully discriminating probable Alzheimer's dementia from vascular dementia and major depression. The Journal of Neuropsychiatry and Clinical Neurosciences 14: 197-201.

35. Kjelvik G, Saltvedt I, White LR, Stenumgård P, Sletvold O, et al. (2014) The brain structural and cognitive basis of odor identification deficits in mild cognitive impairment and Alzheimer's disease. BMC Neurol 14: 168.

36. Vasavada MM, Wang J, Eslinger PJ, Gill DJ, Sun X, et al. (2015) Olfactory cortex degeneration in Alzheimer's disease and mild cognitive impairment. J Alzheimers Dis 45: 947-958.

37. Cain WS, Stevens JC, Nickou CM, Giles A, Johnston I, et al. (1995) Life-span development of odor identification, learning, and olfactory sensitivity. Perception 24: 1457-1472.

38. Hummel T, Rissom K, Reden J, Hähner A, Weidenbecher M, et al. (2009) Effects of olfactory training in patients with olfactory loss. The Laryngoscope 119: 496-499.

39. Pekala K, Chandra RK, Turner JH (2016) Efficacy of olfactory training in patients with olfactory loss: a systematic review and meta-analysis. Int Forum Allergy Rhinol 6: 299-307.

40. Wysocki CJ, Dorries KM, Beauchamp GK (1989) Ability to perceive androstenone can be acquired by ostensibly anosmic people. Proc Natl Acad Sci USA 86: 7976-7978.

41. Barnard ND, Bush AI, Ceccarelli A, Cooper J, de Jager CA, et al. (2014) Dietary and lifestyle guidelines for the prevention of Alzheimer's disease. Neurobiology of Aging 35: S74-S78.

42. Jimbo D, Kimura Y, Taniguchi M, Inoue M, Urakami K (2009) Effect of aromatherapy on patients with Alzheimer's disease. Psychogeriatrics 9: 173-179.

43. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, et al. (2006) Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Annals of Internal Medicine 144: 73-81.

44. Radak Z, Hart N, Sarga L, Koltai E, Atalay M, et al. (2010) Exercise plays a preventive role against Alzheimer's disease. J Alzheimers Dis 20: 777-783.

45. Solfrizzi V, Panza F, Frisardi V, Seripa D, Logroscino G, et al. (2011) Diet and Alzheimer's disease risk factors or prevention: the current evidence. Expert Rev Neurother 11: 677-708.

46. Jackman AH, Doty RL (2005) Utility of a three-item smell identification test in detecting olfactory dysfunction. The Laryngoscope 115: 2209-2212.

Supplemental Table 1: Quantitative smell tests currently in use.

Test name	Number of items/ different odors	Internet source
Smell Identification Test (UPSIT) [1,2]	40 different odors	https://sensonics.com/smell-identification-test-international-versions-available.html
Snap & Sniff Olfactory Test System [3]	20 items test (Threshold)	https://sensonics.com/snapandsniffolfactorytests.html
Sniffin' Sticks Identification Test 16 [4-6]	16 different odors	https://smelltest.eu/en/product/burghart-sniffin-sticks-identification-test-16-blue/
Screening 12 Test [7,8]	12 different odors	http://smelltest.eu/en/product/buy-burghart-sniffin-sticks-smelltest-pens/
Brief Smell Identification Test [9,10]	12 different odors	https://sensonics.com/brief-smell-identification-test.html
Odor Discrimination/Memory Test [11,12]	12 items test using 4 different odors	https://sensonics.com/smell-products/odor-memory-test.html
The Pediatric Smell Wheel [13]	11 different odors	https://sensonics.com/smell-products/the-pediatric-smell-wheel.html
NIH Toolbox Odor Identification Test [14]	9 different odors	http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox/intro-to-nih-toolbox/sensation
Smell Diskettes [15]	8 different odors	http://www.smelldiskettes.com/en/gebrauchsanweisung.php
4-Item NHANES Pocket Smell Test [16]	4 different odors	https://sensonics.com/smell-products/pocket-smell-test-50.html
Quick Smell Identification Test [17]	3 different odors	https://sensonics.com/smell-products/quick-smell-identification-test.html
Pocket Smell Test [18]	3 different odors	https://sensonics.com/smell-products/pocket-smell-test.html

Supplemental Table References

1. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, et al. (1984) Smell identification ability: changes with age. Science 226: 1441-1443.
2. Doty RL, Shaman P, Kimmelman CP, Dann MS (1984) University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. Laryngoscope 94: 176-178.
3. Jiang RS, Liang KL (2018) A Pilot Study of the Snap & Sniff Threshold Test. Ann Otol Rhinol Laryngol 127: 312-316.
4. Lawton M, Hu MT, Baig F, Ruffmann C, Barron E, et al. (2016) Equating scores of the University of Pennsylvania Smell Identification Test and Sniffin' Sticks test in patients with Parkinson's disease. Parkinsonism Relat Disord 33: 96-101.
5. Kang W, Dong F, Li D, Quinn TJ, Chen S, et al. (2016) The predictive value of SS-16 in clinically diagnosed Parkinson's disease patients: comparison with (99m)Tc-TRODAT-1 SPECT scans. Transl Neurodegener 5: 15.
6. Bastos LO, Guerreiro MM, Lees AJ, Warner TT, Silveira-Moriyama L (2015) Effects of age and cognition on a cross-cultural paediatric adaptation of the Sniffin' Sticks Identification Test. PLoS One 10: e0131641.
7. Eibenstein A, Fioretti AB, Lena C, Rosati N, Ottaviano I, et al. (2005) Olfactory screening test: experience in 102 Italian subjects. Acta Otorhinolaryngol Ital 25: 18-22.
8. Ottaviano G, Savietto E, Scarpa B, Bertocco A, Maculan P, et al. (2018) Influence of number of drugs on olfaction in the elderly. Rhinology 56: 351-357.
9. Double KL, Rowe DB, Hayes M, Chan DKY, Blackie J, et al. (2003) Identifying the pattern of olfactory deficits in Parkinson disease using the brief smell identification test. Archives of Neurology 60: 545-549.
10. Menon C, Westervelt HJ, Jahn DR, Dressel JA, O'Bryant SE (2013) Normative performance on the Brief Smell Identification Test (BSIT) in a multi-ethnic bilingual cohort: a Project FRONTIER study. Clin Neuropsychol 27: 946-961.
11. Choudhury ES, Moberg P, Doty RL (2003) Influences of age and sex on a microencapsulated odor memory test. Chem Senses 28: 799-805.
12. Doty RL, Tourbier I, Ng V, Neff J, Armstrong D, et al. (2015) Influences of hormone replacement therapy on olfactory and cognitive function in postmenopausal women. Neurobiol Aging 36: 2053-2059.
13. Cameron EL, Doty RL (2013) Odor identification testing in children and young adults using the smell wheel. Int J Pediatr Otorhinolaryngol 77: 346-350.
14. Dalton P, Doty RL, Murphy C, Frank R, Hoffman HJ, et al. (2013) Olfactory assessment using the NIH Toolbox. Neurology 80: S32-S36.
15. Briner HR, Simmen D (1999) Smell diskettes as screening test of olfaction. Rhinology 37: 145-148.
16. Liu G, Zong G, Doty RL, Sun Q (2016) Prevalence and risk factors of taste and smell impairment in a nationwide representative sample of the US population: a cross-sectional study. BMJ Open 6: e013246.
17. Jackman AH, Doty RL (2005) Utility of a three-item smell identification test in detecting olfactory dysfunction. Laryngoscope 115: 2209-2212.
18. Duff K, McCaffrey RJ, Solomon GS (2002) The Pocket Smell Test: successfully discriminating probable Alzheimer's dementia from vascular dementia and major depression. The J Neuropsychiatry Clin Neurosci 14: 197-201.

Appendix B. Original research article based on chapter 5, experiment 4

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RESEARCH ARTICLE

Volumetric MRI Demonstrates Atrophy of the Olfactory Cortex in AD

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Abstract: ***Objective:*** Alzheimer disease (AD) is a chronic neurodegenerative disorder that affects millions of individuals worldwide. Symptoms include memory dysfunction and deficits in attention, planning, language, and overall cognitive function. Olfactory dysfunction is a common symptom of AD and evidence supports that it is an early marker. Furthermore, olfactory bulb and entorhinal cortex atrophy are well described in AD. However, in AD, no studies have assessed the olfactory cortex as a whole and if sex effects are observed.

Methods: Magnetic Resonance Imaging was used to scan 39 participants with an average age of 72 years and included men and women. AAL Single-Subject Atlas (implemented in PNEURO tool - PMOD 3.8) was used to determine the volume of the olfactory cortex and the hippocampus. Olfactory cortex volume was lower in both men and women AD cases compared with controls. This decrease was more apparent in the left olfactory cortex and was influenced by age. As expected, hippocampal volume was also significantly reduced in AD. However, this was only observed in the male cohort. A significant correlation was observed between levels of education and hippocampal volume in controls that were not detected in the AD participants. Asymmetry was observed in the olfactory cortex volume when comparing left and right volumes in both the control and AD participants, which was not observed in the hippocampus.

Results: These data highlight the importance of the role of olfactory cortical atrophy in the pathogenesis of AD and the interplay between the olfactory deficits and degeneration of olfactory regions in the brain.

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1. INTRODUCTION

Numerous health problems occur with aging, such as cognitive decline, cardiac disorders and/or neurodegenerative diseases, including Alzheimer disease (AD) [1]. Neurodegenerative diseases, such as Parkinson disease (PD), AD and Huntington disease (HD), are progressive disorders that lead to neuronal loss as well as structural alterations in the brain [2]. AD is one of the most debilitating types of dementia cases and may contribute up to 60 - 70% of the cases worldwide [3, 4].

Olfactory dysfunction is a common symptom of neurodegenerative disorders, including AD [5, 6]. Indeed, several studies have shown, using age-matched controls, that the sense of smell is affected in the early stage of the disease [7, 8]. Olfactory dysfunction is also observed in individuals with Mild Cognitive Impairment (MCI), some of which will progress to AD [9]. The sense of smell also declines in otherwise healthy elderly people [10]. However, this is accelerated in individuals with a neurological disease. Positive correlations have also been shown between odour identification and cognition scores [11]. These data suggest that levels of olfactory function may be a marker of impending AD and should be assessed, along with other markers, in aging individuals.

Olfactory dysfunction may have a number of causes, such as nose or brain injury, age-related structural alterations in the olfactory system, environmental or biological alterations, which reduce the efficiency of smelling [12]. Based on the olfactory dysfunction observed in AD, it would be predicted that olfactory structures in the brain could be affected [13]. Indeed, AD individuals show atrophy in a number of olfactory brain regions and in the primary olfactory cortex (POC) [14-16]. In AD, atrophy is also observed in other brain regions, including the hippocampus [13], basal forebrain and thalamus [17]. However, in the medial temporal lobe, AD pathology and volume loss appear earliest in the

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entorhinal cortex, an area within the POC [18, 19]. Thus while many studies indicate that hippocampal atrophy is the best core marker for AD, measurements of the entorhinal cortex may provide the earliest detection [17, 20-22].

Interestingly, β-Amyloid burden does not directly affect the olfactory deficits, despite the fact that β-Amyloid is observed in AD brains, and its accumulation in the olfactory bulb is one of the earliest sites of pathology in AD [15, 23-25]. β-Amyloid accumulation is also observed in individuals without AD [26]. In contrast to the β-Amyloid accumulation, neurofibrillary tangles appear to affect olfactory function [6, 24]. These tangles are detected in the olfactory bulb and primary olfactory sensory cortices in human AD brains [16]. Moreover, neurofibrillary tangles in AD are found in the area of olfactory processing [25, 27]. Other molecular phenotypes in the AD olfactory bulb are also observed, including accumulation of Tau proteins (correlates with AD stage) [28] and caspase activation [29]. Caspase-6 activation, which occurs early in the pathogenesis of AD [30, 31], is observed in the AD olfactory bulb and its activity associated with Tau pathology but not β-Amyloid accumulation [29].

Structural and functional brain asymmetries are observed in humans and animals [32, 33]. Of note, in AD patients, POC volume atrophy in both sides of the brain have been reported [14]. However, using metabolic measures such as 18-F-fluorodeoxyglucose-PET or MRI, the left hemispheric alterations in AD are more severe and occur prior to the atrophy in the right hemisphere [33-35]. Aside from the lateral asymmetry, the gray matter concentration in the olfactory system also differs according to sex [36], which supports morphofunctional differences between men and women olfactory neuronal networks.

A number of studies have shown atrophy of the entorhinal cortex in AD [18, 19, 21]. However, there is a lack of literature regarding olfactory cortex (OC) volume in AD, and in particular, whether there are sex-specific effects. In this study, we wished to determine if there is atrophy of the OC in a cohort of AD individuals, and if so, how sex, age and level of education may influence this. Furthermore, we determined if a/symmetry of these brain regions is observed.

2. MATERIALS AND METHODS

2.1. Participants

The samples used in the present case-control study included 25 cognitively healthy older adults (14 women and 11 men) and 14 individuals with a diagnosis of AD (9 women and 5 men) from an observational study previously described in Croteau *et al.* [37]. All participants provided written informed consent prior to study entry. Ethical approval for this study was obtained from the ethics committees of the Research Center on Aging - CIUSSS de l'Estrie - (2009-111 and 2010-163). Probable or possible AD was defined using the National Institute on Aging - Alzheimer's Association (NIA-AA) criteria [38] with or without the use of prescribed medication for AD [39].

2.2. MRI Screening

Participants were assessed using a 1.5 Tesla magnetic resonance imaging scanner (MRI Sonata, Siemens Medical Solutions, Erlangen, Germany). T1-weighted sequence (TR = 16.00ms, TE=4.68ms, field of view = 256 x 240 x 192 mm, matrix size= 256 x 256 x 164, flip angle = 20° and 1mm isotropic voxels) was obtained. Volumetric MR imaging measurements were performed using PNEURO tool implemented in PMOD 3.8 (PMOD Technologies Ltd., Zurich, Switzerland). Brain anatomy was automatically segmented into 119 volumes of interest according to the Automatic Anatomical Labelling atlas (AAL atlas); [40]. We consider the OC region in the brain as defined by J Dejerine [41] and as used in a recent study [42]. The OC includes the olfactory tract, amygdala, piriform cortex, anterior perforated substance, the subcallosal area (including the subcallosal cingulate gyrus) and the anterior cingulate cortex.

2.3. Statistical Analysis

The MRI screening results of the OC and hippocampal volumes, and the assessment of a/symmetry, were first compared using an unpaired *t*-test in order to avoid any bias from covariates as suggested by Hyatt *et al.* [43]. Pearson's correlation tests were used to determine if there was a correlation between age and OC or hippocampal volume in the control and AD participants. Pearson's correlation tests were also used to determine if there was a correlation between MMSE scores and/or education levels within these brain regions. The unpaired *t*-test and correlation analysis were performed using Graphpad Prism 7 (GraphPad Software San Diego, CA, USA). SPSS software (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY) was used for the GLM regression analysis. The raw OC and/or hippocampus volume data was the dependent variable, disease (AD) was the fixed factor and age was a covariate. A 2-way ANCOVA analysis was done in order to assess for interaction effects between sex and disease on OC volume. The level of significance was set at $p < 0.05$.

3. RESULTS

3.1. Demographics

Table 1 shows the demographic information including cognition tests and education levels for the control participants (14 women, 11 men) and AD individuals (9 women, 5 men). There was no significant difference in the gender proportion between the controls and the AD group. There was a subtle yet significant difference in age between controls and AD individuals ($p=0.047$). This was due to a difference in age between the women controls and AD participants ($p=0.03$). There was no difference in age between the male controls and AD participants. As expected, the Mini-Mental State Examination (MMSE) showed significant differences between the two groups ($p<0.0001$). For education levels, there was no significant difference observed between the control or AD participants when men and women are combined, or when separated by sex. However, overall, the men (control and AD) had higher education levels than the women population (control and AD) ($p=0.0004$).

Table 1. Demographic Profiles. MMSE: Mini-Mental State Examination.

-	-	Control (n=25, M=11 ,F=14)				AD(n=14, M=5, F=9)				p
-	-	Min	Max	Mean	SD	Min	Max	Mean	SD	-
Men + women	Age (y)	65	86	71.1	5.22	65	82	75.6	4.60	0.047*
	MMSE score	27	30	29.3	0.89	19	29	25.4	2.96	<0.0001*
	Education (y)	7	22	14.8	4.54	8	22	12.6	4.72	0.181
Men	Age (y)	65	85	71.0	6.68	70	76	72.6	2.41	0.613
	MMSE score	28	30	29.5	0.69	23	29	25.3	2.87	0.0004*
	Education (y)	13	22	17.1	2.88	10	22	17.3	5.12	0.940
Women	Age (y)	65	78	71.2	3.99	65	82	75.6	5.27	0.03*
	MMSE score	27	30	29.1	1.03	19	29	25.4	3.17	0.0005*
	Education (y)	7	19	12.9	4.84	8	16	10.6	2.83	0.200

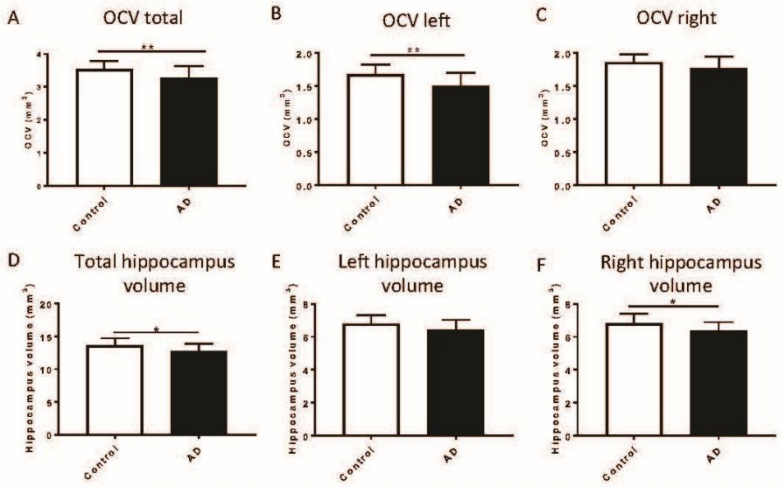

Fig. (1). Olfactory cortex and hippocampal atrophy in AD. A) A significant decrease is observed by MRI in the olfactory cortex total volume in AD compared to control participants (p= 0.009). **B)** A significant decrease is also observed in the olfactory cortex left volume in AD patients (p= 0.003). **C)** A trend decrease is observed in the olfactory cortex right volume in AD patients (p= 0.06). **D)** A significant decrease is observed by MRI in the total hippocampus volume in AD individuals compared to control participants (p= 0.03). **E)** A trend decrease in the hippocampus left volume is detected in AD *vs* controls (p= 0.055). **F)** A significant atrophy is observed in the hippocampus right volume in AD compared to control participants (p= 0.03). Control n=25, AD n=14, men and women combined. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. Atrophy of the Olfactory Cortex in AD

We first wished to assess the OC volumes without confounding the data using covariates as studies have suggested that this may bias the results [43]. The MRI findings demonstrated a significantly lower total OC volume in AD compared to control participants (men and women combined, p=0.009, Fig. **1A**). This is most obvious in the left OC volume (p=0.003, Fig. **1B**) with only a trend decrease in the right OC volume (p=0.06, Fig. **1C**). In order to determine if both sexes demonstrated OC volume atrophy, we assessed this separately for men and women. Despite the small sample size, there was a significant reduction in the total OC volume in men with AD compared to control participants (9.3%, p=0.045). This decrease is more pronounced in the left OC volume compared to the right (left, 11.45%, p=0.02; right, 7.34%, p=0.12). In women AD participants, there was a trend decrease in the total volume (6.8%, p=0.06), and a significant decrease in the left OC volume (10%, p=0.04). The right OC volume showed no change in women between control and AD.

As expected, there was a significant decrease in the total volume of the hippocampus in the AD participants compared

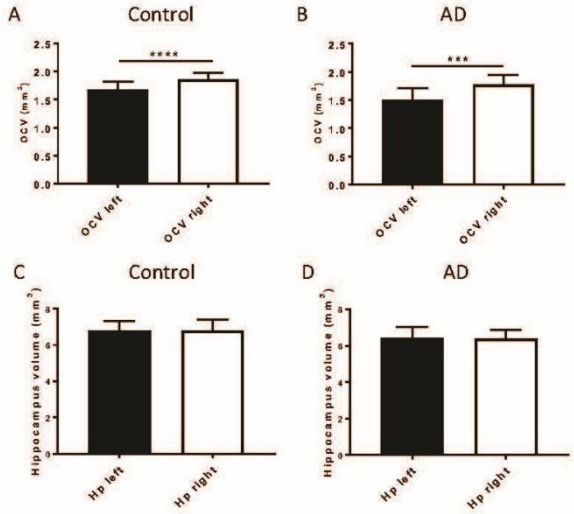

Fig. (2). Select regions of the brain show asymmetry. **A)** A significant difference is observed in the left vs. right olfactory cortex volume of control participants ($p < 0.0001$). **B)** This is also observed in individuals with AD ($p=0.0009$). **C)** In sharp contrast, no asymmetry is observed in the left vs. right hippocampus volume of control participants or in **D)** AD participants. Control n=25, AD n=14, men and women combined. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

to the control group (men and women combined, $p=0.03$, Fig. **1D**). This was more pronounced when comparing the right hippocampal volume ($p=0.03$, Fig. **1F**) than the left hippocampal volume ($p=0.055$, Fig. **1E**). Dissecting out by sex, men AD participants showed a significantly lower total hippocampal volume (9.2%, $p=0.03$) and right hippocampal volume (10.7%, $p=0.011$) compared to controls. For the hippocampal volume in women, no difference was observed between AD and controls in this cohort.

3.3. Region-Specific Brain Asymmetry

Structural, functional and behavioral brain asymmetries are observed in humans for a number of brain regions [33]. However, there is limited data on the OC. A significant asymmetry was observed in our cohort in the OC of controls when comparing the left and the right volumes ($p<0.0001$, Fig. **2A**). We noted a similar asymmetry in the OC of the AD participants ($p=0.0009$, Fig. **2B**). In both groups, the left OC volume was significantly lower than the right side (9.8% in the control group and 15.1% in AD patients). This asymmetry was observed in both the men ($p=0.003$) and women ($p=0.0009$) control participants, and in the women AD participants ($p=0.005$). However, it was not detected in men with AD. In all cases where asymmetry was observed, the left OC volume was smaller compared to the right.

In sharp contrast to the asymmetry observed in the OC volume, no asymmetry was observed in our cohort in the hippocampus when comparing left *versus* right volumes in the control group (Fig. **2C**) or in the AD participants (Fig. **2D**). A similar result was found when the groups were separated based on sex.

3.4. Age Associated with Olfactory Cortex Volume in AD

We first assessed if there was a correlation between left or right OC volume and age in controls and AD. Comparing the slopes of the left and right OC volume in the control participants in relation to age (men and women combined) revealed no significant difference between the slopes (Fig. **3A**). A similar finding was observed in the AD participants (men and women combined, Fig. **3B**). We then assessed age in relation to the left OC volume between the control and AD groups. There was a significant difference between the slopes suggesting that the pathogenesis of AD affects the relationship between the OC volume and age ($p=0.03$, Fig. **3C**). The rate of the OC volume decrease with age was higher in the AD compared to the control participants. This was not observed when comparing age and the volume of the right OC in control and AD participants (Fig. **3D**).

We next performed a general linear model (GLM) regression analysis in order to determine if age and/or sex impacts the relationship between OC volume and disease. In the unadjusted model, there was a significant effect of disease on the total ($p=0.009$) and left ($p=0.003$) OC volumes. This relationship held when adjusted for age (total OCV, $p=0.03$; left OCV $p=0.012$) or sex (total OCV, $p=0.015$; left OCV, $p=0.010$; right OCV, $p=0.04$) (Table **2**). When men and women were analyzed separately, the relationship between OCV and disease held despite adjusting for age (men: total OCV, $p=0.04$; left OCV, $p=0.014$; women, total OCV, $p=0.03$, left, $p=0.02$, Table **2**). The results of the regression analysis demonstrate that disease is a significant predictor of OC volume. In order to rule out any interaction effects between sex and disease, we next performed a 2-way ANCOVA.

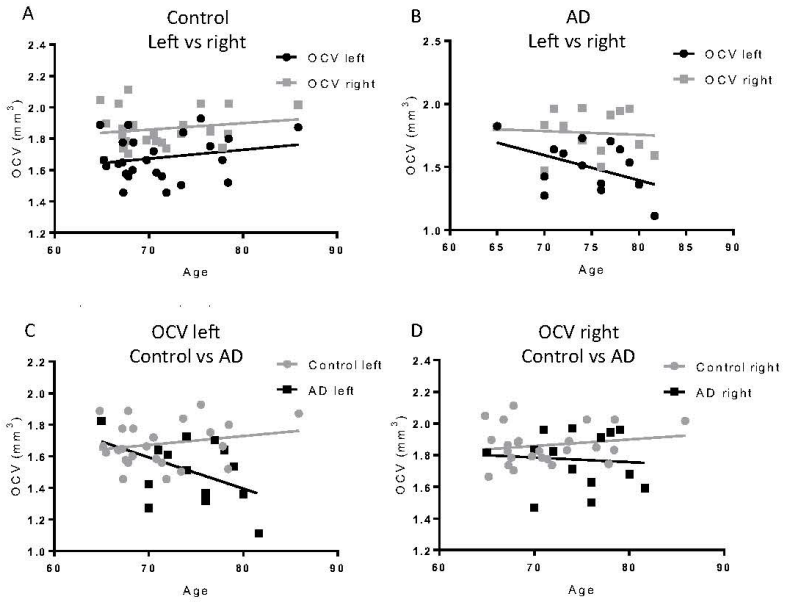

Fig. (3). Aging influences left olfactory cortical volume in AD. A) There is no significant correlation in controls between the left olfactory cortex volume (r=0.2099, r^2=0.0441, p=0.314) or the right (r=0.1803, r^2=0.032, p=0.389) and age. Furthermore, there is no significant difference between the slopes of the left *vs.* right olfactory cortical volume in control participants with age. **B)** In AD participants, there is no significant correlation between the left olfactory cortex volume (r=0.4526, r^2=0.205, p=0.104) or right (r=0.1803, r^2=0.006, p=0.795) and age. Moreover, there is no significant difference between the slopes of the left *vs.* right olfactory cortical volume in AD participants with age. **C)** However, there is a significant difference in the slopes of the left olfactory cortical volume with age in control compared to AD (p=0.03). **D)** No such difference is observed in the right olfactory cortex volume between control and AD with age. Control n=25, AD n=14; men and women combined. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 2. GLM regression analysis of olfactory cortical volume and disease.

-	OCV Total			OCV Left			OCV Right		
-	**Variables**	**F**	p	**Variables**	**F**	p	**Variables**	**F**	p
Women	**Unadjusted**	-	-	**Unadjusted**	-	-	**Unadjusted**	-	-
	(F=3.731, R^2=0.151, p=0.067)		-	(F=4.632, R^2=0.181, p=0.043*)		-	(F=1.801, R^2=0.079, p=0.194)		-
	Disease	3.731	0.067	Disease	4.632	0.043*	Disease	1.801	0.194
	Adjusted for the Age		-	**Adjusted for the Age**		-	**Adjusted for the Age**		-
	(F=4.015, R^2=0.286, p=0.034*)		-	(F=4.903, R^2=0.329, p=0.019*)		-	(F=1.981, R^2=0.165, p=0.164)		-
	Disease	0.944	0.343	Disease	1.300	0.019*	Disease	0.348	0.562
	Age	3.801	0.065	Age	4.420	0.048*	Age	2.070	0.166
Men	**Unadjusted**	-	-	**Unadjusted**	-	-	**Unadjusted**	-	-
	(F=4.825, R^2=0.256, p=0.0,045*)		-	(F=6.826, R^2=0.328, p=0.02*)		-	(F=2.724, R^2=0.163, p=0.121)		-
	Disease	4.825	0.045*	Disease	6.826	0.02*	Disease	2.724	0.121
	Adjusted for the Age		-	**Adjusted for the Age**		-	**Adjusted for the Age**		-

Table 2 contd…

-	OCV Total			OCV Left			OCV Right		
-	**Variables**	**F**	p	**Variables**	**F**	p	**Variables**	**F**	p
	(F=4.229, R^2=0.394, p=0.038*)		-	(F=5.985, R^2=0.479, p=0.014*)		-	(F=2.397, R^2=0.269, p=0.130)		-
	Disease	6.547	0.024*	Disease	9.613	0.008*	Disease	3.516	0.083
	Age	2.958	0.109	Age	3.786	0.074	Age	1.895	0.192
Men and Women	**Unadjusted**	-	-	**Unadjusted**	-	-	**Unadjusted**	-	-
	(F=7.669, R^2=0.172,p=0.009*)		-	(F=10.199, R^2=0.216, p=0.003*)		-	(F=3.680, R^2=0.090, p=0.063)		-
	Disease	7.669	0.009*	Disease	10.199	0.003*	Disease	3.680	0.063
	Adjusted for the Age		-	**Adjusted for the Age**		-	**Adjusted for the Age**		-
	(F=3.731, R^2=0.172, p=0.034*)		-	(F=5.041, R^2=0.219, p=0.012*)		-	(F=1.895, R^2=0.095, p=0.165)		-
	Disease	6.721	0.014*	Disease	8.281	0.007*	Disease	3.752	0.061
	Age	0.000	0.99	Age	0.124	0.727	Age	0.190	0.666
	Adjusted for the Sex		-	**Adjusted for the Sex**		-	**Adjusted for the Sex**		-
	(F=4.763, R2=0.209, p=0.015*)		-	(F=5.309, R2=0.228, p=0.010*)		-	(F=3.622, R2=0.168, p=0.037*)		-
	Disease	8.365	0.006*	Disease	10.388	0.003*	Disease	4.490	0.041*
	Sex	1.710	0.199	Sex	0.544	0.465	Sex	3.332	0.076

Table 3. Analysis of interaction between disease and sex using two-way analysis of covariance (ANCOVA).

OCV Total				OCV Left				OCV Right			
Variables		**F**	p	-	**Variables**	**F**	p	-	**Variables**	**F**	p
Adjusted for the Sex			-	**Adjusted for the Sex**			-	**Adjusted for the Sex**			-
(F=3.154, R2=0.213, p=0.037*)			-	(F=3.452, R2=0.228, p=0.027*)				(F=2.510, R2=0.177, p=0.075)			-
-	Disease	8.257	0.007*	-	Disease	9.805	0.004*	-	Disease	4.802	0.035*
-	Sex	1.828	0.185	-	Sex	0.549	0.464	-	Sex	3.681	0.063
-	Disease*sex	0.159	0.693	-	Disease*sex	0.001	0.874	-	Disease*sex	0.405	0.528
Adjusted for the Sex with Age as Covariate			-	**Adjusted for the Sex with Age as Covariate**			-	**Adjusted for the Sex with Age as Covariate**			-
(F=2.310, R2=0.214, p=0.078)			-	(F=2.583, R2=0.233, p=0.054)				(F=1.835, R2=0.178, p=0.145)			-
-	Disease	7.104	0.012*	-	Disease	8.081	0.008*	-	Disease	4.705	0.042*
-	Sex	1.815	0.187	-	Sex	0.633	0.432	-	Sex	3.404	0.074
-	Age	0.038	0.846	-	Age	0.211	0.649	-	Age	0.022	0.883
-	Disease*sex	0.173	0.680	-	Disease*sex	0.047	0.829	-	Disease*sex	0.363	0.551

The result of this analysis demonstrated that there is no interaction between sex and diagnosis on OC volume and suggests that the decrease in OC volume that is observed in the AD cases are independent of sex effects (total, p=0.04; left p=0.03, Table **3**). However, when the model is adjusted for sex with age as covariate (and including interaction), the model no longer holds (Table **3**). Despite this, disease still remains a significant predictor of OC volume (total OC volume, p=0.012; left p=0.0.008).

3.5. Levels of Education Influence the Volume of the Hippocampus in Controls

In order to determine if aging influences hippocampal volume we first performed a Pearson's correlation test. We found no correlation between age and the total volume of the hippocampus in the control population (Fig. **4A**) or in the AD group (Fig. **4B**). Furthermore, no difference was detected when comparing the slopes of the left or right volumes and age in control *vs*. AD (Fig. **4C**, **D**). We next assessed if

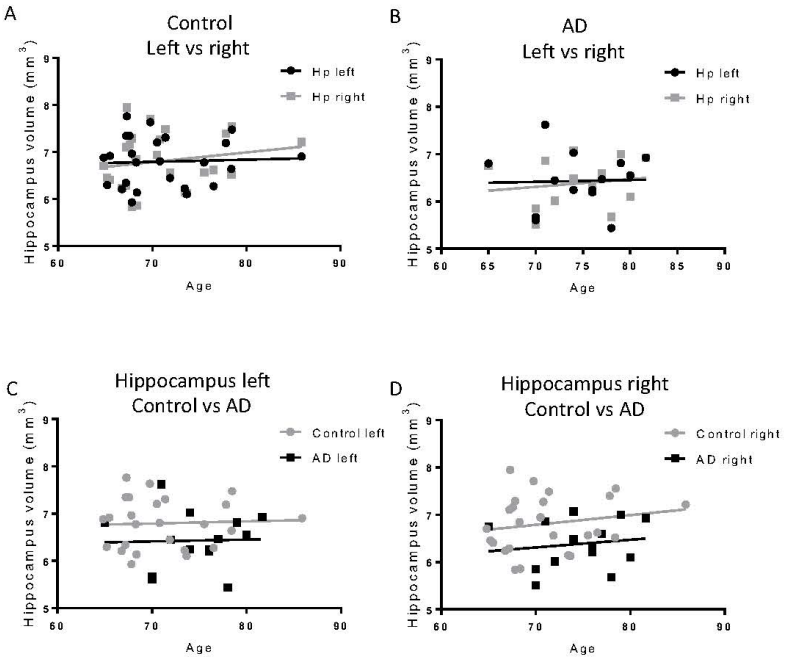

Fig. (4). Aging does not influence the volume of the hippocampus. A) There is no significant correlation in controls between the left hippocampus volume (r=0.0463, r^2=0.002, p=0.826) or right (r=1832, r^2=0.033, p=0.381) and age. Furthermore, there is no significant difference between the slopes of the left vs. right hippocampus volume in control participants with age (p=0.611). **B)** A similar result is seen in AD cases (left hippocampus volume (r=0.0298, r^2=0.0009, p=0.919); right (r=0.1466, r^2=0.021, p=0.617). Moreover, there is no significant difference between the slopes of the left vs. right hippocampus volume in AD participants with aging in (p =0.804). **C**) There is no significant difference between the slopes of the left hippocampus volume with age in control compared to AD (p=0.986). **D)** Similarly, no significant difference is detected between the slopes of the right hippocampus volume with age in control compared to AD (p= 0.917). Control n=25, AD n=14; men and women combined. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

age or sex impacts the relationship between total hippocampal volume and disease using GLM regression analysis. In the unadjusted model, the disease has an effect on hippocampal volume as expected (p=0.03). When the model was adjusted for age, this effect was lost. However, the disease was still a significant predictor of hippocampal volume (p=0.03). When the model was adjusted for sex, including disease sex interactions, the results demonstrated that disease impacts hippocampal volumes (total hippocampal volume, p=0.002). When separating the sexes, in men, the relationship held when adjusting for age only when assessing the right hippocampal volume (p=0.03). In women, no relationship was observed between disease and hippocampal volume.

We next assessed whether years of education impact OC or hippocampal volumes. There was no correlation observed between education levels and OC volume in control or AD participants (data not shown). However, there was a positive correlation in the controls (men and women combined) between levels of education and total hippocampal volume (Fig. **5A**, r=0.4125, R^2=0.170, p=0.04), left (Fig. **5C**, r=0.4132, R^2=0.171, p=0.04) and trend in the right (Fig. **5D**, r=0.3955, R^2=0.156, p=0.0504). In the control participants, higher levels of education correlated with hippocampal volume. In sharp contrast, this was not observed in the AD group (men and women combined, Fig. **5A-C**).

4. DISCUSSION

The results from the MRI assessment of this cohort demonstrate that both OC and hippocampal atrophy are observed in the AD participants when the sexes are combined. However, when the data are separated by sex, while the OC atrophy was observed in both sexes, the hippocampal atrophy was only observed in men with AD and not women. In addition, the olfactory cortex showed asymmetry in both the control and AD group. In contrast, a comparison of the left and right volumes of the hippocampus showed no such asymmetry for either group. GLM regression analysis demonstrated that the predominant effect on the OC volume in the AD participants was due to the disease and not age or sex. Despite a significant association between years of education and the volume of the hippocampus in controls, no correla-

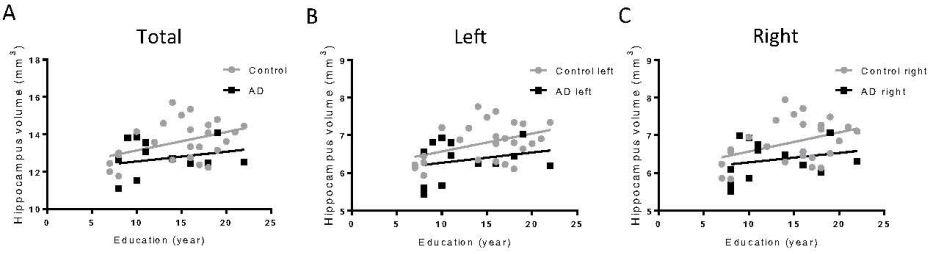

Fig. (5). Levels of education influences the volume of the hippocampus. A) There is positive correlation between the total volume of the hippocampus and education levels in the control participants (r=0.4125, r^2=0.170, p=0.04). Of note, no such correlation is observed between hippocampus volume and education levels in AD (r=0.2477, r^2=0.061, p=0.415). However, there is no significant difference between the slopes of the total hippocampus volume and education levels in control and AD participants. **B)** There is positive correlation between hippocampus left volume and education levels in control participants (r=0.4132, r^2=0.171, p=0.04) that is not found in AD (r=0.2509, r^2=0.062, p=0.408). There is no significant difference between the slopes of the left hippocampus volume with education in control compared to AD. **C)** No correlation is observed between education levels and the right hippocampus volume in the control group (r=0.3955, r^2=0.156, p=0.0504) or in the in the AD group (r=0.2319, r^2=0.0538, p=0.446). Furthermore, the difference between the slopes of education levels and right hippocampus volume of control and AD is not significant. Control n=25, AD n=13; men and women combined. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tion was observed with MMSE scores and the volumes of the OC or hippocampus.

Numerous articles demonstrate olfactory dysfunction is observed in the majority of AD cases [5, 8, 12] and that this is an early symptom of the disease [7, 44]. Furthermore, a number of publications show atrophy of olfactory brain regions occurs in AD. This includes structures such as the olfactory bulb [15, 23, 45], olfactory tract [15], olfactory epithelium [16], piriform cortex [46, 47] and entorhinal cortex [18, 48]. Olfactory dysfunction and olfactory brain region atrophy are also observed in MCI [18, 49] and evidence would suggest that this is progressive [50]. However, no studies have specifically assessed the overall OC volume in AD and few studies have assessed the sexes independently despite the fact that more women are affected with AD than men. Our study demonstrated that OC volume was decreased in AD, and despite the low n, when separating by sex, we saw this effect in both men and women. Furthermore, the results of the regression analysis demonstrate that AD is a significant predictor of OC volume and that the decrease in OC volume that is observed in the AD cases is independent of sex effects. Our studies showed that the left OC was more affected than the right OC in AD. This is concurrent with other evidence demonstrating that, in general, left-brain regions are more affected in AD than the right [51-53].

As expected [54], we also observed hippocampal atrophy in the AD cases. This was most pronounced in the right hippocampus with only a trend decrease in the left hippocampal volume as previously observed [34, 55, 56]. However, this contrasts with other studies showing increased atrophy in the left hippocampus in AD [53, 57, 58]. This finding could be the result of the association between the right hippocampus volume and stress [59], depression [60] and/or other factors that influence hippocampal volume, including exercise, age, genetic factors or handedness (all participants involved in our cohort were right-handed). Hippocampal volume is also influenced by sex. The reasons for this include hormones and/or hormone therapy, patterns of brain development, psychosocial stress responses, longevity and/or inflammatory reaction differences between men and women [61]. Surprisingly, when our results were analyzed by sex, we only observed hippocampal atrophy in the male AD cases, despite the n being lower in this group compared to the women. There was a subtle but significant age difference between our women control and AD cases that was not present in our male cohort. However, the female AD cases were slightly older, not younger, than the controls, which would have been expected to make the difference more pronounced. It is possible that the women AD cases in our cohort represent the hippocampal sparing subgroup of AD, which demonstrates a less aggressive progression [62]. Protocols for identifying this particular subgroup are still lack in the clinic. While actual atrophy per se and relative sparing of pathology in the hippocampus is observed in these cases, in general, there is increased neurofibrillary tangles in other regions including the nucleus basalis of Meynert and cortical areas [63, 64].

In general, the human brain shows functional (brain networks and activity, neurochemical, behavioral) and structural (brain regions, cytoarchitectural, dendritic arborization) asymmetry [33]. In particular, several brain regions, including the frontal lobe, the occipital pole, cortical thickness, gray matter volume in the frontal lobe and the anterior insular cortex exhibit asymmetry. Furthermore, sex-dependent brain asymmetry is also common. Conflicting results have been found for the entorhinal olfactory cortex, with some studies showing asymmetry [65] and others not [34]. The significant asymmetry observed in the left vs. right OC volumes in our control population could be the result of development, environment, gender, pathological factors or hereditary influences [33]. Of note, in our control cohort, we observed that both men and women presented with OC asymmetry. Regarding the OC volume asymmetry in the AD cases, a number of studies demonstrate that left-brain regions are more affected in MCI and/or AD [51-53, 66, 67]. While

the reasons for this are still somewhat unclear, a number of hypotheses have been put forward to explain this, including genetics and disease risk factors such as Apolipoprotein E allele 4 (APOE4). Carriers of the APOE4 allele(s) may have specific regional differences in the left brain regions [67-70], and the evidence suggests that the APOE protein may be involved in brain development and the physiological and/or pathological patterns observed later in life [34]. It has also been shown that olfactory function correlates with the volume of olfactory brain regions and that there are left *vs*. right nostril correlations with the size of the olfactory bulb, which would impact the piriform cortex, a primary olfactory brain region. In line with our results of atrophy in the OC in the AD cases, a study has shown that the distance to the left nostril for detecting an odor is significantly shorter in AD and MCI participants compared to controls [71]. In contrast to the OC, we did not observe an asymmetry in the hippocampus in the control cohort or in the AD participants. A similar finding was observed when separating by sex. Conflicting results have been observed regarding a/symmetry of the hippocampus in AD cases, with some observing asymmetry [72, 73] and others showing similar findings to ours [34, 74]. This might be dependent upon the stage of the disease. Asymmetry in left vs. right hippocampal volumes has been observed at baseline in AD patients. However, this was not observed in follow-up scans 15 months later, suggesting that the asymmetry was reduced as the disease progresses [52].

Age is the highest risk factor for AD and indeed, our results demonstrate age does impact OC volume in the AD participants. However, it is important to note that irrespective of age, there is an impact of the disease on the OC volume. Aging affects brain size, vasculature, olfaction and cognition [75]. Underlying molecular changes have also been shown, including alterations in neurotransmitters, hormones, neurotropic factors, and reactive oxygen species, among others [75].

Higher education levels, healthy diets and exercise have been linked to better cognitive functioning later in life [75]. Indeed, Nobel *et al*. demonstrate a dose-dependent effect of education on hippocampal volume in cognitively healthy individuals [76]. In agreement with other studies [76], we noted an association between education levels and the volume of the hippocampus in the controls. This was not detected in the AD cases despite no significant difference in education levels between the control and AD participants. Reduced levels of education have been linked to increased exposure to life stress, which is associated with hippocampal structural differences [76]. A number of environmental factors may affect brain development including, poverty, exposure to violence, family turmoil and instability [76, 77] thus it cannot be excluded that this affect is solely due to number of years of education in our control cohort. However, cognitive reserve has been demonstrated to confirm benefit on brain structure and function and that may provide protection against neurodegeneration [76, 78, 79].

There is insufficient literature regarding the volume of the OC in MCI or AD patients. The majority of the literature has focused on the olfactory bulb and entorhinal cortex. Our study suggests that other regions in the OC may be similarly affected. This stands to reason as the olfactory cortex receives projections from the olfactory bulb as well as being involved in feedback pathways [80, 81].

CONCLUSION

Our study suggests that the volume of the OC may be used as a disease measure in AD, thus saving considerable time with regards to the segmentation analysis necessary to obtain olfactory brain regions such as the piriform and/or entorhinal cortex. Additional studies are required to establish a sufficient baseline for the volume of the OC in control and MCI/AD cases in order to further validate these data. Moreover, more research is required to understand the links between the early olfactory dysfunction observed in neurodegenerative diseases including MCI, AD PD and HD and how this impacts the structures in the olfactory regions of the brain and *vice versa*.

LIST OF ABBREVIATIONS

AD	=	Alzheimer Disease
HD	=	Huntington Disease
ICV	=	Intracranial Volume
MCI	=	Mild Cognitive Impairment
MMSE	=	Mini-Mental State Examination
MRI	=	Magnetic Resonance Imaging
OC	=	Olfactory Cortex
PD	=	Parkinson Disease
POC	=	Primary Olfactory Cortex

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for this study was obtained from the ethics committees of the Research Center on Aging - CIUSSS de l'Estrie, Canada (2009-111 and 2010163).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All human procedures were followed in accordance with the guidelines of the Declaration of Helisnsiki.

CONSENT FOR PUBLICATION

Written Informed consent was obtained from the study participants.

AVAILABILITY OF DATA AND MATERIALS

Not Applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Cermakova P, Eriksdotter M, Lund LH, Winblad B, Religa P, Religa D. Heart failure and Alzheimer's disease. J Intern Med 2015; 277(4): 406-25. http://dx.doi.org/10.1111/joim.12287 PMID: 25041352

[2] Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol 2008; 29(8): 357-65. http://dx.doi.org/10.1016/j.it.2008.05.002 PMID: 18599350

[3] de la Monte SM, Wands JR. Alzheimer-associated neuronal thread protein mediated cell death is linked to impaired insulin signaling. J Alzheimers Dis 2004; 6(3): 231-42. http://dx.doi.org/10.3233/JAD-2004-6304 PMID: 15201478

[4] Niikura T, Tajima H, Kita Y. Neuronal cell death in Alzheimer's disease and a neuroprotective factor, humanin. Curr Neuropharmacol 2006; 4(2): 139-47. http://dx.doi.org/10.2174/157015906776359577 PMID: 18615127

[5] Doty RL, Perl DP, Steele JC, *et al.* Olfactory dysfunction in three neurodegenerative diseases. Geriatrics 1991; 46(1): 47-51. PMID: 1894145

[6] Ruan Y, Zheng XY, Zhang HL, Zhu W, Zhu J. Olfactory dysfunctions in neurodegenerative disorders. J Neurosci Res 2012; 90(9): 1693-700. http://dx.doi.org/10.1002/jnr.23054 PMID: 22674288

[7] Djordjevic J, Jones-Gotman M, De Sousa K, Chertkow H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 2008; 29(5): 693-706. http://dx.doi.org/10.1016/j.neurobiolaging.2006.11.014 PMID: 17207898

[8] Godoy MD, Voegels RL, Pinna FdeR, Imamura R, Farfel JM. Olfaction in neurologic and neurodegenerative diseases: a literature review. Int Arch Otorhinolaryngol 2015; 19(2): 176-9. PMID: 25992176

[9] Morris JC, Storandt M, Miller JP, *et al.* Mild cognitive impairment represents early-stage Alzheimer disease. Arch Neurol 2001; 58(3): 397-405. http://dx.doi.org/10.1001/archneur.58.3.397 PMID: 11255443

[10] Zhang C, Wang X. Initiation of the age-related decline of odor identification in humans: a meta-analysis. Ageing Res Rev 2017; 40: 45-50. http://dx.doi.org/10.1016/j.arr.2017.08.004 PMID: 28830800

[11] Larsson M, Finkel D, Pedersen NL. Odor identification: influences of age, gender, cognition, and personality. J Gerontol B Psychol Sci Soc Sci 2000; 55(5): 304-10. http://dx.doi.org/10.1093/geronb/55.5.P304 PMID: 10985295

[12] Doty RL. Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? Lancet Neurol 2017; 16(6): 478-88. http://dx.doi.org/10.1016/S1474-4422(17)30123-0 PMID: 28504111

[13] Pearson RC, Esiri MM, Hiorns RW, Wilcock GK, Powell TP. Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. Proc Natl Acad Sci USA 1985; 82(13): 4531-4. http://dx.doi.org/10.1073/pnas.82.13.4531 PMID: 3859874

[14] Vasavada MM, Wang J, Eslinger PJ, *et al.* Olfactory cortex degeneration in Alzheimer's disease and mild cognitive impairment. J Alzheimers Dis 2015; 45(3): 947-58. http://dx.doi.org/10.3233/JAD-141947 PMID: 25633674

[15] Thomann PA, Dos Santos V, Seidl U, Toro P, Essig M, Schröder J. MRI-derived atrophy of the olfactory bulb and tract in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 2009; 17(1): 213-21. http://dx.doi.org/10.3233/JAD-2009-1036 PMID: 19494444

[16] Arnold SE, Lee EB, Moberg PJ, *et al.* Olfactory epithelium amyloid-beta and paired helical filament-tau pathology in Alzheimer disease. Ann Neurol 2010; 67(4): 462-9. http://dx.doi.org/10.1002/ana.21910 PMID: 20437581

[17] Pini L, Pievani M, Bocchetta M, *et al.* Brain atrophy in Alzheimer's Disease and aging. Ageing Res Rev 2016; 30: 25-48. http://dx.doi.org/10.1016/j.arr.2016.01.002 PMID: 26827786

[18] Devanand DP, Bansal R, Liu J, Hao X, Pradhaban G, Peterson BS. MRI hippocampal and entorhinal cortex mapping in predicting conversion to Alzheimer's disease. Neuroimage 2012; 60(3): 1622-9. http://dx.doi.org/10.1016/j.neuroimage.2012.01.075 PMID: 22289801

[19] Du AT, Schuff N, Kramer JH, *et al.* Higher atrophy rate of entorhinal cortex than hippocampus in AD. Neurology 2004; 62(3): 422-7. http://dx.doi.org/10.1212/01.WNL.0000106462.72282.90 PMID: 14872024

[20] Dubois B, Feldman HH, Jacova C, *et al.* Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 2007; 6(8): 734-46. http://dx.doi.org/10.1016/S1474-4422(07)70178-3 PMID: 17616482

[21] Johnson KA, Fox NC, Sperling RA, Klunk WE. Brain imaging in Alzheimer disease. Cold Spring Harb Perspect Med 2012; 2(4): a006213. http://dx.doi.org/10.1101/cshperspect.a006213 PMID: 22474610

[22] Henneman WJ, Sluimer JD, Barnes J, *et al.* Hippocampal atrophy rates in Alzheimer disease: added value over whole brain volume measures. Neurology 2009; 72(11): 999-1007. http://dx.doi.org/10.1212/01.wnl.0000344568.09360.31 PMID: 19289740

[23] Christen-Zaech S, Kraftsik R, Pillevuit O, *et al.* Early olfactory involvement in Alzheimer's disease. Can J Neurol Sci 2003; 30(1): 20-5. http://dx.doi.org/10.1017/S0317167100002389 PMID: 12619779

[24] Wilson RS, Schneider JA, Arnold SE, Tang Y, Boyle PA, Bennett DA. Olfactory identification and incidence of mild cognitive impairment in older age. Arch Gen Psychiatry 2007; 64(7): 802-8. http://dx.doi.org/10.1001/archpsyc.64.7.802 PMID: 17606814

[25] Bahar-Fuchs A, Chételat G, Villemagne VL, *et al.* Olfactory deficits and amyloid-β burden in Alzheimer's disease, mild cognitive impairment, and healthy aging: a PiB PET study. J Alzheimers Dis 2010; 22(4): 1081-7. http://dx.doi.org/10.3233/JAD-2010-100696 PMID: 20930316

[26] Carvalho DZ, St Louis EK, Knopman DS, *et al.* Association of excessive daytime sleepiness with longitudinal β-amyloid accumulation in elderly persons without dementia. JAMA Neurol 2018; 75(6): 672-80. http://dx.doi.org/10.1001/jamaneurol.2018.0049 PMID: 29532057

[27] Kovács T, Cairns NJ, Lantos PL. Olfactory centres in Alzheimer's disease: olfactory bulb is involved in early Braak's stages. Neuroreport 2001; 12(2): 285-8. http://dx.doi.org/10.1097/00001756-200102120-00021 PMID: 11209936

[28] Lazarov O, Marr RA. Neurogenesis and Alzheimer's disease: at the crossroads. Exp Neurol 2010; 223(2): 267-81. http://dx.doi.org/10.1016/j.expneurol.2009.08.009 PMID: 19699201

[29] Foveau B, Albrecht S, Bennett DA, Correa JA, LeBlanc AC. Increased Caspase-6 activity in the human anterior olfactory nuclei of the olfactory bulb is associated with cognitive impairment. Acta Neuropathol Commun 2016; 4(1): 127. http://dx.doi.org/10.1186/s40478-016-0400-x PMID: 27931265

[30] LeBlanc AC. Caspase-6 as a novel early target in the treatment of Alzheimer's disease. Eur J Neurosci 2013; 37(12): 2005-18. http://dx.doi.org/10.1111/ejn.12250 PMID: 23773070

[31] Albrecht S, Bourdeau M, Bennett D, Mufson EJ, Bhattacharjee M, LeBlanc AC. Activation of caspase-6 in aging and mild cognitive impairment. Am J Pathol 2007; 170(4): 1200-9. http://dx.doi.org/10.2353/ajpath.2007.060974 PMID: 17392160

[32] Derflinger S, Sorg C, Gaser C, *et al.* Grey-matter atrophy in Alzheimer's disease is asymmetric but not lateralized. J Alzheimers Dis 2011; 25(2): 347-57.
http://dx.doi.org/10.3233/JAD-2011-110041 PMID: 21422522
[33] Toga AW, Thompson PM. Mapping brain asymmetry. Nat Rev Neurosci 2003; 4(1): 37-48.
http://dx.doi.org/10.1038/nrn1009 PMID: 12511860
[34] Donix M, Burggren AC, Scharf M, *et al.* APOE associated hemispheric asymmetry of entorhinal cortical thickness in aging and Alzheimer's disease. Psychiatry Res 2013; 214(3): 212-20.
http://dx.doi.org/10.1016/j.pscychresns.2013.09.006 PMID: 24080518
[35] Thompson PM, Hayashi KM, Dutton RA, *et al.* Tracking Alzheimer's disease. Ann N Y Acad Sci 2007; 1097: 183-214.
http://dx.doi.org/10.1196/annals.1379.017 PMID: 17413023
[36] Garcia-Falgueras A, Junque C, Giménez M, Caldú X, Segovia S, Guillamon A. Sex differences in the human olfactory system. Brain Res 2006; 1116(1): 103-11.
http://dx.doi.org/10.1016/j.brainres.2006.07.115 PMID: 16942757
[37] Croteau E, Castellano CA, Fortier M, *et al.* A cross-sectional comparison of brain glucose and ketone metabolism in cognitively healthy older adults, mild cognitive impairment and early Alzheimer's disease. Exp Gerontol 2018; 107: 18-26.
http://dx.doi.org/10.1016/j.exger.2017.07.004 PMID: 28709938
[38] McKhann GM, Knopman DS, Chertkow H, *et al.* The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7(3): 263-9.
http://dx.doi.org/10.1016/j.jalz.2011.03.005 PMID: 21514250
[39] Castellano CA, Paquet N, Dionne IJ, *et al.* A 3-month aerobic training program improves brain energy metabolism in mild alzheimer's disease: preliminary results from a neuroimaging study. J Alzheimers Dis 2017; 56(4): 1459-68.
http://dx.doi.org/10.3233/JAD-161163 PMID: 28157102
[40] Rolls ET, Joliot M, Tzourio-Mazoyer N. Implementation of a new parcellation of the orbitofrontal cortex in the automated anatomical labeling atlas. Neuroimage 2015; 122: 1-5.
http://dx.doi.org/10.1016/j.neuroimage.2015.07.075 PMID: 26241684
[41] Anatomie des Centres Nerveux. Am J Psychiatry 1895; 51(3): 402-3.
http://dx.doi.org/10.1176/ajp.51.3.402
[42] Fjaeldstad A, Fernandes HM, Van Hartevelt TJ, *et al.* Brain fingerprints of olfaction: a novel structural method for assessing olfactory cortical networks in health and disease. Sci Rep 2017; 7: 42534.
http://dx.doi.org/10.1038/srep42534 PMID: 28195241
[43] Hyatt CS, Owens MM, Crowe ML, Carter NT, Lynam DR, Miller JD. The quandary of covarying: a brief review and empirical examination of covariate use in structural neuroimaging studies on psychological variables. Neuroimage 2020; 205: 116225.
http://dx.doi.org/10.1016/j.neuroimage.2019.116225 PMID: 31568872
[44] Conti MZ, Vicini-Chilovi B, Riva M, *et al.* Odor identification deficit predicts clinical conversion from mild cognitive impairment to dementia due to Alzheimer's disease. Arch Clin Neuropsychol 2013; 28(5): 391-9.
http://dx.doi.org/10.1093/arclin/act032
[45] Buschhüter D, Smitka M, Puschmann S, *et al.* Correlation between olfactory bulb volume and olfactory function. Neuroimage 2008; 42(2): 498-502.
http://dx.doi.org/10.1016/j.neuroimage.2008.05.004 PMID: 18555701
[46] Li W, Howard JD, Gottfried JA. Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease. Brain 2010; 133(9): 2714-26.
http://dx.doi.org/10.1093/brain/awq209 PMID: 20724290
[47] Harper L, Bouwman F, Burton EJ, *et al.* Patterns of atrophy in pathologically confirmed dementias: a voxelwise analysis. J Neurol Neurosurg Psychiatry 2017; 88(11): 908-16.
http://dx.doi.org/10.1136/jnnp-2016-314978 PMID: 28473626
[48] Juottonen K, Laakso MP, Insausti R, *et al.* Volumes of the entorhinal and perirhinal cortices in Alzheimer's disease. Neurobiol Aging 1998; 19(1): 15-22.
http://dx.doi.org/10.1016/S0197-4580(98)00007-4 PMID: 9562498
[49] Du AT, Schuff N, Amend D, *et al.* Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. J Neurol Neurosurg Psychiatry 2001; 71(4): 441-7.
http://dx.doi.org/10.1136/jnnp.71.4.441 PMID: 11561025
[50] Westervelt HJ, Bruce JM, Coon WG, Tremont G. Odor identification in mild cognitive impairment subtypes. J Clin Exp Neuropsychol 2008; 30(2): 151-6.
http://dx.doi.org/10.1080/13803390701287408 PMID: 18938667
[51] Loewenstein DA, Barker WW, Chang JY, *et al.* Predominant left hemisphere metabolic dysfunction in dementia. Arch Neurol 1989; 46(2): 146-52.
http://dx.doi.org/10.1001/archneur.1989.00520380046012 PMID: 2783845
[52] Barnes J, Scahill RI, Schott JM, Frost C, Rossor MN, Fox NC. Does Alzheimer's disease affect hippocampal asymmetry? Evidence from a cross-sectional and longitudinal volumetric MRI study. Dement Geriatr Cogn Disord 2005; 19(5-6): 338-44.
http://dx.doi.org/10.1159/000084560 PMID: 15785035
[53] Zhao W, Wang X, Yin C, He M, Li S, Han Y. Trajectories of the hippocampal subfields atrophy in the Alzheimer's disease: a structural imaging study. Front Neuroinform 2019; 13: 13.
http://dx.doi.org/10.3389/fninf.2019.00013 PMID: 30983985
[54] Mueller SG, Schuff N, Yaffe K, Madison C, Miller B, Weiner MW. Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. Hum Brain Mapp 2010; 31(9): 1339-47.
http://dx.doi.org/10.1002/hbm.20934 PMID: 20839293
[55] Apostolova LG, Dinov ID, Dutton RA, *et al.* 3D comparison of hippocampal atrophy in amnestic mild cognitive impairment and Alzheimer's disease. Brain 2006; 129(Pt 11): 2867-73.
http://dx.doi.org/10.1093/brain/awl274 PMID: 17018552
[56] Laakso MP, Lehtovirta M, Partanen K, Riekkinen PJ, Soininen H. Hippocampus in Alzheimer's disease: a 3-year follow-up MRI study. Biol Psychiatry 2000; 47(6): 557-61.
http://dx.doi.org/10.1016/S0006-3223(99)00167-5 PMID: 10715362
[57] Murphy C, Jernigan TL, Fennema-Notestine C. Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: a structural MRI study. J Int Neuropsychol Soc 2003; 9(3): 459-71.
http://dx.doi.org/10.1017/S1355617703930116 PMID: 12666770
[58] Pievani M, Galluzzi S, Thompson PM, Rasser PE, Bonetti M, Frisoni GB. APOE4 is associated with greater atrophy of the hippocampal formation in Alzheimer's disease. Neuroimage 2011; 55(3): 909-19.
http://dx.doi.org/10.1016/j.neuroimage.2010.12.081 PMID: 21224004
[59] Lindgren L, Bergdahl J, Nyberg L. Longitudinal evidence for smaller hippocampus volume as a vulnerability factor for perceived stress. Cereb Cortex 2016; 26(8): 3527-33.
http://dx.doi.org/10.1093/cercor/bhw154 PMID: 27230217
[60] Mathias SR, Knowles EE, Kent JW Jr, *et al.* Recurrent major depression and right hippocampal volume: a bivariate linkage and association study. Hum Brain Mapp 2016; 37(1): 191-202.
http://dx.doi.org/10.1002/hbm.23025 PMID: 26485182
[61] Fisher DW, Bennett DA, Dong H. Sexual dimorphism in predisposition to Alzheimer's disease. Neurobiol Aging 2018; 70: 308-24.
http://dx.doi.org/10.1016/j.neurobiolaging.2018.04.004 PMID: 29754747
[62] Ferreira D, Verhagen C, Hernández-Cabrera JA, *et al.* Distinct subtypes of Alzheimer's disease based on patterns of brain atrophy: longitudinal trajectories and clinical applications. Sci Rep 2017; 7: 46263.
http://dx.doi.org/10.1038/srep46263 PMID: 28417965
[63] Hanna Al-Shaikh FS, Duara R, Crook JE, *et al.* Selective vulnerability of the nucleus basalis of meynert among neuropathologic subtypes of Alzheimer disease. JAMA Neurol 2020; 77(2): 225-33.
PMID: 31657834
[64] Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurol 2011; 10(9): 785-96.
http://dx.doi.org/10.1016/S1474-4422(11)70156-9 PMID: 21802369
[65] Kong V, Devenyi GA, Gallino D, *et al.* Early-in-life neuroanatomical and behavioural trajectories in a triple transgenic model of Alzheimer's disease. Brain Struct Funct 2018; 223(7): 3365-82.
http://dx.doi.org/10.1007/s00429-018-1691-4 PMID: 29948190
[66] Yu HL, Chen ZJ, Zhao JW, Duan SR, Zhao JK. Olfactory impairment and hippocampal volume in a Chinese MCI clinical sample. Alzheimer Dis Assoc Disord 2019; 33(2): 124-8.
http://dx.doi.org/10.1097/WAD.0000000000000305 PMID: 31094709

[67] Liang X, Yin Z, Liu R, *et al.* The role of MRI biomarkers and their interactions with cognitive status and APOE ε4 in nondemented elderly subjects. Neurodegener Dis 2018; 18(5-6): 270-80. http://dx.doi.org/10.1159/000495754 PMID: 30673663

[68] Kelly DA, Seidenberg M, Reiter K, *et al.* Differential 5-year brain atrophy rates in cognitively declining and stable APOE-ε4 elders. Neuropsychology 2018; 32(6): 647-53. http://dx.doi.org/10.1037/neu0000444 PMID: 29911873

[69] Low A, Ng KP, Chander RJ, Wong B, Kandiah N. Association of asymmetrical white matter hyperintensities and apolipoprotein E4 on cognitive impairment. J Alzheimers Dis 2019; 70(3): 953-64. http://dx.doi.org/10.3233/JAD-190159 PMID: 31306121

[70] Abraham M, Seidenberg M, Kelly DA, *et al.* Episodic memory and hippocampal volume predict 5-year mild cognitive impairment conversion in healthy apolipoprotein ε4 carriers. J Int Neuropsychol Soc 2020; 26(7): 733-8. http://dx.doi.org/10.1017/S1355617720000181 PMID: 32131913

[71] Stamps JJ, Bartoshuk LM, Heilman KM. A brief olfactory test for Alzheimer's disease. J Neurol Sci 2013; 333(1-2): 19-24. http://dx.doi.org/10.1016/j.jns.2013.06.033 PMID: 23927938

[72] Nobis L, Manohar SG, Smith SM, *et al.* Hippocampal volume across age: Nomograms derived from over 19,700 people in UK Biobank. Neuroimage Clin 2019; 23: 101904. http://dx.doi.org/10.1016/j.nicl.2019.101904 PMID: 31254939

[73] Pedraza O, Bowers D, Gilmore R. Asymmetry of the hippocampus and amygdala in MRI volumetric measurements of normal adults. J Int Neuropsychol Soc 2004; 10(5): 664-78. http://dx.doi.org/10.1017/S1355617704105080 PMID: 15327714

[74] Geroldi C, Laakso MP, DeCarli C, *et al.* Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer's disease: a volumetric MRI study. J Neurol Neurosurg Psychiatry 2000; 68(1): 93-6. http://dx.doi.org/10.1136/jnnp.68.1.93 PMID: 10601411

[75] Peters R. Ageing and the brain. Postgrad Med J 2006; 82(964): 84-8. http://dx.doi.org/10.1136/pgmj.2005.036665 PMID: 16461469

[76] Noble KG, Grieve SM, Korgaonkar MS, *et al.* Hippocampal volume varies with educational attainment across the life-span. Front Hum Neurosci 2012; 6: 307. http://dx.doi.org/10.3389/fnhum.2012.00307 PMID: 23162453

[77] Evans GW. The environment of childhood poverty. Am Psychol 2004; 59(2): 77-92. http://dx.doi.org/10.1037/0003-066X.59.2.77 PMID: 14992634

[78] Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc 2002; 8(3): 448-60. http://dx.doi.org/10.1017/S1355617702813248 PMID: 11939702

[79] Stern Y. Cognitive reserve and Alzheimer disease. Alzheimer Dis Assoc Disord 2006; 20(3)(Suppl. 2): S69-74. http://dx.doi.org/10.1097/00002093-200607001-00010 PMID: 16917199

[80] Wilson DA, Kadohisa M, Fletcher ML. Cortical contributions to olfaction: plasticity and perception. Semin Cell Dev Biol 2006; 17(4): 462-70. http://dx.doi.org/10.1016/j.semcdb.2006.04.008 PMID: 16750923

[81] Wilson DA, Stevenson RJ. The fundamental role of memory in olfactory perception. Trends Neurosci 2003; 26(5): 243-7. http://dx.doi.org/10.1016/S0166-2236(03)00076-6 PMID: 12744840

Appendix C. Permission to use the published papers for thesis/ dissertation purposes

Fwd: article permission

Ambreen Irshad <ambreenirshad@benthamscience.net>
Sun 05-Sep-21 3:38 PM
To: biomajed@hotmail.com <biomajed@hotmail.com>

Grant of Permission

Dear Dr. Al-Otaibi :

Thank you for your interest in our copyrighted material, and for requesting permission for its use.

Permission is granted for the following subject to the conditions outlined below:

Curr Alzheimer Res . 2020;17(10):904-915.
doi: 10.2174/1567205017666201215120909.
Volumetric MRI demonstrates atrophy of the olfactory cortex in AD
Majed Al-Otaibi 1, Melissa Lessard-Beaudoin 2, Christian-Alexandre Castellano 2, Denis Gris 3, Stephen C Cunnane 2, Rona K Graham 2

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Sincerely,

AMBREEN IRSHAD
***Permissions & Rights* Manager**
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Email: ambreenirshad@benthamscience.net

Note:

Please reply to this email at ambreenirshad@benthamscience.net otherwise your email will not reach me.

---------- Forwarded message ---------
From: **Current Alzheimer Research CAR** <car@benthamscience.net>

Appendix D. Copy of ethics granted for experiments 1 and 2 in chapter 4

Approbation finale
Dossier 2015-477

Centre de santé et de services sociaux –
Institut universitaire de gériatrie
de Sherbrooke

Sherbrooke, le 16 février 2015

Professeure Rona Graham, Ph. D.
FMSS Département de physiologie et biophysique
Université de Sherbrooke
2500, boul. de l'Université
Sherbrooke, QC J1K 2R1

Objet : **Approbation finale du projet de recherche**
Le rôle de la dysfonction olfactive dans le déclin cognitif observé dans le vieillissement de la population
Dossier 2015-477

Professeure Graham,

Le Comité d'éthique de la recherche du CSSS-IUGS accuse réception des documents suivants, déposés sur Nagano, à la suite de l'approbation conditionnelle du projet cité en rubrique :

- Formulaires de consentement modifiés en date du 11 février 2015;
- Cartes postales modifiées en date du 11 février 2015.

Comme les modifications apportées à ces documents ont été jugées satisfaisantes, j'ai le plaisir de vous informer que votre projet de recherche a été approuvé.

La présente approbation éthique est valide pour un an à compter du 16 février 2015, date de l'approbation finale. Un mois avant la date d'échéance, vous devrez faire une demande de renouvellement auprès du comité d'éthique de la recherche du CSSS-IUGS en utilisant le document du comité prévu à cet effet. Les formulaires pourront être complétés à partir du logiciel Nagano, disponible à l'adresse suivante : nagano.csss-iugs.ca .

En acceptant le certificat d'éthique joint en annexe, vous vous engagez à :

- Soumettre, pour approbation préalable au comité, toute demande de modification au projet de recherche ou à tout document approuvé par le comité pour la réalisation de votre projet.
- Soumettre, dès que cela est porté à votre connaissance et s'il y a lieu :
  - les réactions indésirables graves, les réactions indésirables et inattendues et les accidents observés en cours de recherche, et ce, dans les six jours ouvrables qui suivent;
  - tout nouveau renseignement sur des éléments susceptibles d'affecter l'intégrité ou l'éthique du projet de recherche ou d'accroître les risques et les inconvénients des sujets, de nuire au bon déroulement du projet ou d'avoir une incidence sur le désir d'un sujet de recherche de continuer sa participation au projet de recherche;
  - toute modification constatée au chapitre de l'équilibre clinique à la lumière des données recueillies;
  - la cessation prématurée du projet de recherche, qu'elle soit temporaire ou permanente;
  - tout problème identifié par un tiers, lors d'une enquête, d'une surveillance ou d'une vérification interne ou externe;

Hôpital et centre d'hébergement D'Youville
Comité d'éthique de la recherche
du CSSS-IUGS

1036, rue Belvédère Sud, Sherbrooke (Québec) J1H 4C4
Téléphone : 819 780-2220, poste 45386
Télécopieur : 819 829-7141

- toute suspension ou annulation de l'approbation octroyée par un organisme de subvention ou de réglementation;
- toute procédure en cours de traitement d'une plainte ou d'une allégation de manquement à l'intégrité ou à l'éthique ainsi que des résultats de la procédure.

La présente décision peut être suspendue ou révoquée en cas de non-respect de ces exigences. En plus du suivi administratif d'usage, le CÉR pourra effectuer un suivi actif au besoin selon les modalités qu'il juge appropriées.

En terminant, nous vous rappelons que vous devez conserver pour une période d'au moins un an suivant la fin du projet, un répertoire distinct comprenant les noms, prénoms, coordonnées, date du début et de fin de la participation de chaque sujet de recherche.

Le Comité d'éthique de la recherche du CSSS-IUGS est institué par le ministre de la Santé et des Services sociaux aux fins de l'application de l'article 21 du Code civil du Québec et respecte les règles émises par l'Énoncé de politique des trois conseils et les Bonnes pratiques cliniques de la CIH.

Je vous prie d'accepter, Professeure Graham, mes meilleures salutations.

Chantal Doré, Ph. D.
Présidente

CD/lv

p. j. Certificat éthique
Formulaires de consentement approuvés
Cartes postales approuvées
Lettres de recrutement approuvées

Hôpital et centre d'hébergement D'Youville
Comité d'éthique de la recherche
du CSSS-IUGS

1036, rue Belvédère Sud, Sherbrooke (Québec) J1H 4C4
Téléphone : 819 780-2220, poste 45386
Télécopieur : 819 829-7141

Appendix E. Copy of ethics granted for experiment 3 and 5 in chapter 4 and 5

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

Professor Annalena Venneri
The University of Sheffield
Department of Neuroscience
Sheffield
S10 2RX

West of Scotland REC 5
West of Scotland Research Ethics Service
Ground Floor, Ward 11
Dykebar Hospital
Grahamston Road
Paisley PA2 7DE
www.nhsggc.org.uk
Date 21 November 2019
Re-issued 13 March 2020
Direct line 0141 314 0211
E-mail WOSREC5@ggc.scot.nhs.uk

Please note: This is an acknowledgement letter from the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

Dear Professor Venneri

Study title:	**Optimising detection of cognitive decline and associated symptoms**
REC reference:	**19/WS/0177**
IRAS project ID:	**244064**

Thank you for your email dated 18th November 2019. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 15 November 2019

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Participant information sheet (PIS) [Patient information sheet]	2	18 November 2019
Participant information sheet (PIS) [Patient information sheet - Highlighted]	2	18 November 2019
Response to Additional Conditions Met [Email]		18 November 2019

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
GP/consultant information sheets or letters [General practitioner information sheet]	1	30 September 2019
IRAS Application Form [IRAS_Form_29102019]		29 October 2019
Participant consent form [Patient consent form]	1	23 October 2019
Participant information sheet (PIS) [Patient information sheet]	2	18 November 2019

Participant information sheet (PIS) [Patient information sheet - Highlighted]	2	18 November 2019
Research protocol or project proposal [Ethics protocol]	1	25 October 2019
Response to Additional Conditions Met [Email]		18 November 2019
Summary CV for Chief Investigator (CI) [Annalena Venneri - CV]	1	30 September 2019
Summary CV for student [Laura Wright - CV]	1	30 September 2019
Summary CV for student [Jose' Manuel Valera Bemejo - CV]	1	30 September 2019
Summary CV for student [Ronan O'Marley - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Matteo De Marco - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Daniel Blackburn - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Ptolemaios Sarriggianis - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Riccardo Manca - CV]	1	30 September 2019

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

19/WS/0177 **Please quote this number on all correspondence**

Yours sincerely

Liz Jamieson

Liz Jamieson
REC Manager

Copy to: HRA.Approval@nhs.net

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Professor Annalena Venneri
The University of Sheffield
Department of Neuroscience
Sheffield
S10 2RX

Email: hra.approval@nhs.net
HCRW.approvals@wales.nhs.uk

21 November 2019

Dear Professor Venneri

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Optimising detection of cognitive decline and associated symptoms**
IRAS project ID:	**244064**
REC reference:	**19/WS/0177**
Sponsor	**Sheffield Teaching Hospitals NHS Foundation Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **244064**. Please quote this on all correspondence.

Yours sincerely,
Rekha Keshvara

Approvals Manager

Email: hra.approval@nhs.net

Copy to:

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
GP/consultant information sheets or letters [General practitioner information sheet]	1	30 September 2019
IRAS Application Form [IRAS_Form_29102019]		29 October 2019
IRAS Application Form XML file [IRAS_Form_29102019]		29 October 2019
IRAS Checklist XML [Checklist_29102019]		29 October 2019
Letter from funder		04 January 2019
Participant consent form [Patient consent form]	1	23 October 2019
Participant information sheet (PIS) [Patient information sheet]	2	18 November 2019
Participant information sheet (PIS) [Patient information sheet - Highlighted]	2	18 November 2019
Research protocol or project proposal [Ethics protocol]	1	25 October 2019
Response to Additional Conditions Met [Email]		18 November 2019
Summary CV for Chief Investigator (CI) [Annalena Venneri - CV]	1	30 September 2019
Summary CV for student [Laura Wright - CV]	1	30 September 2019
Summary CV for student [Jose' Manuel Valera Bemejo - CV]	1	30 September 2019
Summary CV for student [Ronan O'Marley - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Matteo De Marco - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Daniel Blackburn - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Ptolemaios Sarriggianis - CV]	1	30 September 2019
Summary CV for supervisor (student research) [Riccardo Manca - CV]	1	30 September 2019

IRAS project ID	244064

Information to support study set up

The below provides all parties with information to support the arranging and confirming of capacity and capability with participating NHS organisations in England and Wales. This is intended to be an accurate reflection of the study at the time of issue of this letter.

Types of participating NHS organisation	Expectations related to confirmation of capacity and capability	Agreement to be used	Funding arrangements	Oversight expectations	HR Good Practice Resource Pack expectations
There is one participating NHS organisation taking part in the study in England. Therefore, there is one site type undertaking the research activities as detailed in the study protocol	This is a single site study sponsored by the participating NHS organisation. You should work with your sponsor R&D office to make arrangements to set up the study. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.	An Organisation Information Document has been submitted and the sponsor is not requesting and does not expect any other site agreement to be used	The study is funded by the Neurocare.	A Principal Investigator is expected to be in place at the participating NHS sites.	Where existing contractual or healthcare placement arrangements are not already in place it is expected that postgraduate students are supervised under close clinical supervision, when undertaking activities that may have a direct bearing on the quality of care, by a clinical supervisor who is an NHS employee or an HEI employee with an honorary clinical or research contract. Where a postgraduate student is undertaking research and clinical supervision is not available or not appropriate, the student should obtain an letter of access. Evidence of standard DBS and barred list checks with occupational health clearance would be expected to support a

					research passport application for letter of access where obtained.

Other information to aid study set-up and delivery

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales in study set-up.
The applicant has indicated that they intend to apply for inclusion on the NIHR CRN Portfolio.

Appendix F. Copy of ethics granted for experiment 4 in chapter 5

Centre intégré universitaire de santé et de services sociaux de l'Estrie – Centre hospitalier universitaire de Sherbrooke
Québec

Sherbrooke, le 23 septembre 2019

Dr Éric Turcotte
FMSS Département de médecine nucléaire et radiobiologie
Université de Sherbrooke

Objet : Approbation d'une demande de renouvellement annuel par le Comité d'éthique de la recherche du CIUSSS de l'Estrie - CHUS

Projet #2009-133, 08-111
Métabolisme cérébral du 11C-acétoacétate chez l'humain.

Bonjour Dr Turcotte,

La présente est pour vous informer que nous avons reçu le formulaire de demande de renouvellement annuel (F9 - 26604) pour le projet mentionné ci-haut.

Une nouvelle approbation a été émise par le Comité d'éthique de la recherche du CIUSSS de l'Estrie – CHUS via révision accélérée et sera valide du 30 septembre 2019 au 30 septembre 2020.

Il est à noter qu'aucun membre du comité d'éthique participant à l'évaluation et à l'approbation de ce projet n'est impliqué dans celui-ci.

Attestation du CÉR (REBA) : En ce qui concerne ce projet de recherche, à titre de représentant du Comité d'éthique de la recherche du CIUSSS de l'Estrie - CHUS, je certifie que:

1. La composition de ce comité d'éthique satisfait aux exigences pertinentes prévues dans le titre 5 de la partie C du Règlement sur les aliments et drogues.
2. Le comité d'éthique de la recherche exerce ses activités de manière conforme aux bonnes pratiques cliniques.
3. Ce comité d'éthique a examiné et approuvé le formulaire de consentement et le protocole d'essai clinique qui sera mené par le chercheur susmentionné, au lieu d'essai indiqué. L'approbation et les opinions du présent comité ont été consignées par écrit.
4. Ce Comité est conforme aux normes américaines. (FWA #00005894 et IRB #00003849)

Espérant le tout à votre convenance, je vous prie d'agréer, Dr Turcotte, mes salutations distinguées.

Dre Annabelle Cumyn, MDCM, MHPE
Présidente du CÉR du CIUSSS de l'Estrie - CHUS

Centre intégré
universitaire de santé
et de services sociaux
de l'Estrie – Centre
hospitalier universitaire
de Sherbrooke
Québec

Sherbrooke, le 29 mars 2017

Dre Nancy Paquet
Service de médecine nucléaire

Objet : Approbation d'une demande de renouvellement annuel par le Comité d'éthique de la recherche du CIUSSS de l'Estrie - CHUS

Projet #2010-163, 09-043
Projet pilote évaluant l'impact de l'exercice physique sur la maladie d'Alzheimer légère chez des sujets sédentaires: quantification avec l'imagerie TEP au 18F-FDG.

Bonjour Dre Paquet,

La présente est pour vous informer que nous avons reçu le formulaire de demande de renouvellement annuel (FCRC/RC9 - 11430) pour le projet mentionné ci-haut.

Une nouvelle approbation a été émise par le Comité d'éthique de la recherche du CIUSSS de l'Estrie - CHUS via révision accélérée et sera valide du **31 mars 2017** jusqu'au **31 mars 2018**.

Il est à noter qu'aucun membre du comité d'éthique participant à l'évaluation et à l'approbation de ce projet n'est impliqué dans celui-ci.

En ce qui concerne l'essai clinique visé, à titre de représentant du Comité d'éthique de la recherche, je certifie que:

1. La composition de ce comité d'éthique satisfait aux exigences pertinentes prévues dans le titre 5 de la partie C du Règlement sur les aliments et drogues.
2. Le comité d'éthique de la recherche exerce ses activités de manière conforme aux bonnes pratiques cliniques, et
3. Ce comité d'éthique a examiné et approuvé le formulaire de consentement et le protocole d'essai clinique qui sera mené par le chercheur susmentionné, au lieu d'essai indiqué. L'approbation et les opinions du présent comité ont été consignées par écrit.
4. Ce Comité est conforme aux normes américaines. (FWA #00005894 et IRB00003849)

Espérant le tout à votre convenance, je vous prie d'agréer, Dre Paquet, mes salutations distinguées.

Annabelle Cumyn, MDCM, MHPE
Co-Présidente du Comité d'éthique de la recherche du
CIUSSS de l'Estrie - CHUS

Appendix G. Acknowledgment of funding appendix ORCA study

The NuAge study was supported by a research grant from the Canadian Institutes of Health Research (CIHR; MOP-62842). The NuAge Database and Biobank are supported by the Fonds de recherche du Québec (FRQ; 2020-VICO-279753), the Quebec Network for Research on Aging, a thematic network funded by the Fonds de Recherche du Québec - Santé (FRQS) and by the Merck-Frosst Chair funded by La Fondation de l'Université de Sherbrooke. The CIMA-Q is supported by the *Fonds d'Innovation Pfizer - Fonds de Recherche Québec – Santé sur la maladie d'Alzheimer et les maladies apparentées* (Pfizer-FRQS; #27239), and we thank their financial support for the completion of the 2014-2017 follow-up calls of NuAge participants. The work in this study was supported by a grant from the Fondation Université de Sherbrooke Merck Sharpe & Dohme, a pilot grant from the Research Centre on Aging CSSS-IUGS, and, in part, by the Canada Research chairs (CRC) program.