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Lexical-Semantic parameters as robust endophenotypes of abnormal cognitive decline in ageing

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Abstract

The objective of this dissertation was to characterise the relative contribution of genetic influences to individual differences in cross sectional performance and decline of semantic-lexical abilities and to investigate whether these linguistic effects indicating semantic degradation are sensitive indicators of medial temporal atrophy in early Alzheimer's disease and in patients with mild cognitive impairment of amnestic type (aMCI).

The effect of ApoE status in the genetic profile of these groups on deterioration of semantic abilities was studied to verify whether there was any relationship between variation in lexical factors and genetic variability. Oral generation of words belonging to two categories (animal and fruits) during a fluency tasks was required.

In AD patients there was an effect of genotype but, although strong, this was diluted by the advanced cognitive deterioration and could only be seen as a tendency to be stronger in ε4 carriers. The words produced by the aMCI carriers were significantly earlier acquired than those of non-carriers and controls. These behavioural findings confirmed evidence from other recent studies and showed that a significant proportion of phenotype variability in performance on fluency tasks was influenced by genetic factors. Impairments in semantic tasks in the ε4 allele carrier population might indicate either that individuals who will develop AD never fully develop semantic skills, or that the neuroanatomical substrate of semantic abilities is selective sensitive to the earliest effects of the AD neuropathology.

On the basis of this result it seemed reasonable to hypothesise that the presence of the "semantic endophenotype" in people carriyng the ApoE vulnerability

mutation might be associated with atrophy in areas early affected by neuropathology due to AD and involved in semantic memory retrieval. Using lexical semantic competency in aMCI carriers as an endophenotype, grey matter volume loss in aMCI ε4 carriers/non-carriers and in controls was compared and the residual volume correlated with allele burden and with age of acquisition values for words produced in a category fluency task. Direct group comparisons showed that carriers had grey matter volume loss which was generally confined to limbic regions and medial temporal structures, and non-carriers had greater atrophy in temporal and parieto-occipital neocortex. aMCI subjects had significantly impoverished lexical semantic output compared to controls, more marked in aMCI carriers. A voxel based correlation analysis showed that greater volume loss in parahippocampal gyrus and thalamus was associated with a tendency to retrieve earlier acquired words in the category fluency task. The results suggest a relatively specific impact of ApoE ε4 burden and underline the value of linguistic assessment in preclinical diagnosis. The detrimental role of this mutation found in aMCI individuals was also assessed at the larger stage in the disease process by direct comparisons in minimal to mild AD ε4 carriers/non-carrier patients. VBM comparison analysis confirmed the observation done in the genetically determined aMCI subgroups. AD ε4- carriers showed greater atrophy in mediotemporal structures compared to non-carriers whose grey matter volume loss was more widespread in more neocortical areas. Finally, an age, gender and education based norms for AoA, Typicality and Familiarity was built up in order to create a valid psychometric instrument able to detect and monitor subtle semantic deficits in ApoE ε4 carriers over time.

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CONTENTS

CHAPTER 1 Alzheimer's disease

1.1 Epidemiology of neurodegeneration

Alzheimer's disease, together with Parkinson's disease and motor neuron disease, represent the group of degenerative brain disorders most common and costly to society. Epidemiological research of these degenerative brain diseases has recently (the last ten years) changed its focus moving from descriptive studies of the disease to the analysis of risk factors. The purpose of this shift has been to understand better the association between risk associated with gene variants to increase the identification and prevention of these diseases. In this dissertation the focus will be on one of these neurodegenerative diseases: Alzheimer's disease (AD).

1.2 Alzheimer's disease

Originally described by Alois Alzheimer in 1907, AD is a progressive neurodegenerative disorder, clinically characterised by initial impairment in memory but as the disease progresses other cognitive skills also decline. In the later stage of the disease abnormal behaviour, delusion and loss of control of physical abilities also appear.

The diagnosis of AD is guided the by the well-established National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) clinical criteria (Mckhann, Drachman, Folstein, Katzman, Price, et al., 1984): *definitive* is only used for disease confirmed at post mortem examination; *probable* is used when clinical symptoms can be detected and all the other causes of dementia have been ruled out; and *possible* is used for subjects meeting criteria associated with other illnesses that may cause central nervous system dysfunction, such as cardiovascular problem disease, but still show many features of AD. There are not definitive diagnostic tests or biological markers of the disease. However, neuropsychological assessment when associated to technological advances in neuroimaging or biochemistry reasonably increases the accuracy of the diagnosis (Chang & Silverman, 2004). This multidisciplinary investigation increases the accuracy level for the diagnosis of “probable AD”. Only the detection of the histopathological hallmarks of AD, senile plaques (SPs), neurofibrillary tangles (NTs) and progressive loss of synapses and neurons, allow a definitive diagnosis of AD to be made (Bacskai, Klunk, Mathis, & Hyman, 2002; Chui, Tierney, Zarow, Lewis, Sobel, et al., 1993; Mori, 2000). At the anatomical level the hippocampus, entorhinal cortex and nucleus basalis of Meynert, which contain a good proportion of the cholinergic neurons of the brain, show substantial cell loss early in AD. Furthermore degenerative change in the forebrain reduces the level of cholin acetylsferase (ChAT), an enzyme necessary for acetylcholine formation, also occurs early in the cause of the disease and has been related to the early symptoms (Davies & Maloney, 1976; Mayeux, 2003a). However deficits in other neurotramitter systems have also been reported, such as reduced levels of GABA and Dopamine and decreased serotonergic and noradrenergic innervations of the cortex (Adolfsson, Gottfries, Roos, & Winblad, 1979; Mann, Lincoln, Yates, Stamp, & Toper, 1980). These findings suggest that in AD it is not possible to address the changes to only one particular neurotransmitter system. Furthermore, these neurochemical deficits might be the result and not the cause of cellular damage (Glenner, 1989).

1.3 AD frequency

The incidence rate (i.e. the number of new cases among healthy individuals over a specific time), increases from 1% annually among people aged 65 to 70 years to 6% to 8% for people over 85 years old (Burns & Iliffe, 2009; Chandra, Pandav, Dodge, Johnston, Belle, et al., 2001; Del Tredici, Rub, De Vos, Bohl, & Braak, 2002; Di Carlo, Baldereschi, Amaducci, Lepore, Bracco, et al., 2002; Fratiglioni, Launer, Andersen, Breteler, Copeland, et al., 2000; Hebert, Beckett, Scherr, & Evans, 2001; Tang, Cross, Andrews, Jacobs, Small, et al., 2001). The BBC reports that in the UK the number of people affected by dementia has arisen from 400, 000 individuals in 2007 to 820, 000 in 2010 with an estimated 1 milion people affected by 2025 ("Dementia 'losing out' to cancer in funding stakes ", 2010). Demographic variables affect the incident rate. Women have a higher probability of developing AD (Andersen, Launer, Dewey, Letenneur, Ott, et al., 1999; Miech, Breitner, Zandi, Khachaturian, Anthony, et al., 2002; Ruitenberg, van Swieten, Witteman, Mehta, van Duijn, et al., 2002) and the rate of the disease seems to be different in different populations. The risk rises almost three times in Caucasian population, weaker but still significant for African American, Carribbean Hispanich and Latino compared to Whites living in the United States (Gurland, Wilder, Lantigua, Stern, Chen, et al., 1999; Tang, et al., 2001) but not among Africans in their native countries (Ogunniyi, Baiyewu, Gureje, Hall, Unverzagt, et al., 2000). Duration of illness is another factor affecting the prevalence of the disease. Under the age of 65 the disease is rare; however by the age of 85 about 20% have this disease (Bowirrat, Treves, Friedland, & Korczyn, 2001; Stevens, Livingston, Kitchen, Manela, Walker, et al., 2002a; W. Wang, Wu, Cheng, Dai, Ross, et al., 2000). The presence of 1 or 2 copies of the apolipoprotein ε4 allele is another risk factor. In particular the ε4 type of the gene has been found to carry a higher risk of AD, while the ε2 type is believed to offer protection against it (Waring & Rosenberg, 2008). There is evidence that the incidence of disease as well as the risk attributable to specific genetic factors such as ApoE genotype, may vary among ethnic groups. In particular the ε4 allele increases the risk of AD threefold in Caucasian populations, while there is a weak but still significant risk factor for AD in Hispanic, Latino or African-American populations (Farrer, Cupples, Haines, Hyman, Kukull, et al., 1997; Meyer, Tschanz, Norton, Welsh-Bohmer, Steffens, et al., 1998; Tang, Stern, Marder, Bell, Gurland, et al., 1998). Recently a study showed a lower frequency of ApoE ε2 and higher frequency of ApoE ε4 allele in the Mongolian population in China compared to Han Chinese one, suggesting an increase susceptibility of that population to AD (Huriletemuer, Wang, Wang, Wang, Zhang, et al., 2010).

Based on these studies, therefore, the risk of developing AD depends on the incidence rate and the life expectancy from birth. For example the lifetime risk (i.e. the probability of someone of a given age and sex developing a condition during their remaining lifespan) for African Americans and some Hispanic ethnic groups may be higher than that observed in the Framingham study (Rosamond, Flegal, Furie, Go, Greenlund, et al., 2008) whose community-based data were based on a group of North Americans of European descent (12% to 19% incidence rate risk for women over 65 and 6% to 10% for men) (Green, Cupples, Go, Benke, Edeki, et al., 2002; Seshadri & Wolf, 2007; Tang, et al., 2001).

1.4 Pathophysiology of AD

Genetic and neuropathological findings of AD have pointed towards aberrant processing of two proteins, amyloid precurson protein (APP) and tau, as being central molecular events in this disease. The classic neuropathological features of AD specific changes in the brain are neuritic plaques composed of β amyloid fibrils and neurofibrillary tangles composed of tau protein (Huang & Jiang, 2009).

1.4.1 Senile Plaque

Amyloid plaques are mostly made up of a protein called B-amyloid which is it self part of a much larger protein called APP (amyloid precursor protein). APP ismade in the cell, transported to the cell membrane and later broken down. Two major pathways are involved in the breakdown of APP. One pathway is normal and causes no problem. The second results in the changes seen in AD and in some of the other dementias (Hardy, 1997; Selkoe, 1994). The extracellular plaques result from endoproteolysis of APP by two enzymes: β-secretase and y-secretase. Some of the resulting fragments (called peptides) stick together and form a short chain called oligomer. Oligomers are also known as amyloid-beta derived diffusible ligands (ADDL). Oligomers of amyloid beta 42 have been shown to cause problems in the communication between neurons. Amyloid beta 42 also produces tiny fibres, or fibrils. When they stick together they form amyloid plaques. Some of these plaques can insert themselves into the membrane of the neuron cell causing substances outside the cell to leak into it, resulting in further damage. This damage results in a build up of Amyloid beta 42 peptide leading to neuron dysfunction and death.

Reduced amiloyd degradation has also been reported as a possible cause of increased Aβ42 accumulation (Di Fede, Catania, Morbin, Rossi, Suardi, et al., 2009).

1.4.2 Neurofibrillary Tangles

Neurofibrillary changes occur in the perikarya of neurons as intracellular neurofibrillary tangles (NFTs) of which the principal components are a highly phosphorylated form of the microtubule-stabilizing protein tau aggregated to paired helical filaments (PHF) (Goedert, Wischik, Crowther, Walker, & Klug, 1988;Tabaton, Mandybur, Perry, Onorato, Autilio-Gambetti, et al., 1989). The presence of PHFs demonstrates the failure of the neuron to properly maintain its cytoskeleton. These aggregates are also found in dendritites and axons and appear as neuropil threads which have been found to be closely correlated with the distribution and severity of neurofibrillary tangles.

The role of amyloid plaques and neurofibrillary tangles on the functioning of the brain is not fully understood. Most people with Alzheimer's disease show evidence of both plaques and tangles, but a small number of people with Alzheimer's only have plaques and some have only neurofibrillary tangles. People with only plaques show a slower rate of deterioration during their lives. People with neurofibrillary tangles are more likely to be diagnosed with frontotemporal dementia. It is reasonable to postulate that progressive cognitive decline associated to the disease is related to a concomitant progressive development of pathological alterations in the brain, which is more significant for the NFTs than SPs (Braak & Braak, 1991).

In this regard it is interesting to observe that NFTs are always confined to limbic structures such as the transentorhinal cortex, hippocampus, the nucleus basalis of Meynert and the amygdala. These areas are critical for memory function and seem to be vulnerable to the distribution of NFTs during ageing. Some authors posit that there are two NFT stages (low-high limbic and neocortical stage) in which the initial low-high density of NTFs in limbic areas could account for the memory impairment associated with ageing (low stage) and as soon as they become more numerous and start to cluster (high limbic stage) might provide the anatomical substrate for MCI and pre-clinical AD. With the spreading of NFTs all over the neocortical areas the corresponding greater cognitive decline could explain the passage from MCI to severe AD (Arnold, Hyman, Flory, Damasio, & Van Hoesen, 1991; Arriagada, Growdon, Hedley-Whyte, & Hyman, 1992). Assuming this concomitant cognitive-pathological progression, the interval between the initial formation of NFTs and their widespread neocortical distribution in the terminal stages of the dementia may take as long as 50 years (Braak & Braak, 1996; Ohm, Muller, Braak, & Bohl, 1995). There is no genetic linkage between AD and tau-processing abnormalities which lead to NFT formation. Some evidence seems to support a genetic factor in causing abnormal amyloid processing (Hardy, 1997; Selkoe, 1994; Yankner, 1996)

However Aβ plaques tend to have higher density in the association cortex rather than in limbic areas. SPs can be seen even in non-demented elderly individuals and none of the transgenic animal overexpressing the AD causing mutation of AβPP has shown NFT formation (Mesulam, 2000). In summary, the exact role of APP is not yet fully elucidated. The latest theoretical trends concerning sporadic Alzheimer's disease (SAD) implicate the overproduction of APP as an acute response to damage such as vascular damage (Hardy, 2009).

1.5 AD risk factors

Although AD is one of the most common neurodegenerative diseases among the elderly, the aetiology of AD is still not well known. However, there have been improvements in the overall knowledge of this disease and improvements in who is diagnosed, compared to a statement published in a 1981 issue of Biomedical Journal noting that an AD diagnosis was often made “when the patient was older and less intelligent than the doctor” (Millard, 1981).

1.5.1 Demographic factors

1.5.1.1 *Education*

It is now well known that for poorly educated individuals the risk of developing AD is significantly higher than for well-educated people (Morishima-Kawashima, Oshima, Ogata, Yamaguchi, Yoshimura, et al., 2000; Stern, Gurland, Tatemichi, Tang, Wilder, et al., 1994). An association has been suggested between greater development of “cognitive reserve”, which appears to have a protective effect, and increases with more years of formal education (Katzman, 1993). It remains unknown, however, if the level of education achieved is a surrogate for other genetic or environmental effects. For example from the characteristics of autobiographical essays written by Catholic sisters when they were an average 22 years old, it was possible to predict the subsequent cognitive impairment and presence of AD (average onset mean 80 years old) with an extremely high degree of accuracy (Snowdon, Kemper, Mortimer, Greiner, Wekstein, et al., 1996). Moreover Whalley et al., (2000) showed that childhood mental ability scores were lower than average among children who eventually developed AD after the age of 65. These studies appear to suggest that educational achievement reflects early determinants of the disease. It is worth considering the role that differences in the quality of the environment during childhood might also have. Indeed individuals from nurturing, supportive households had a lower risk of subsequent disease when adults (Hall, Gao, Unverzagt, & Hendrie, 2000; Moceri, Kukull, Emanuel, van Belle, & Larson, 2000)

1.5.1.2 ***Age***

One of the strongest risk factor for AD is age. After the age of 65 the frequency of AD has been shown to double approximately every 5 years, with a rate of 3% in the 65-74 age range to a rate of 47% in subjects older than 80 (Bachman, Wolf, Linn, Knoefel, Cobb, et al., 1993; Henderson, 1992). According to the result of a prospective study which used the person-years approach (Breslow & Day, 1987) to calculate age-specific incidence of dementia and AD (that is by dividing the number of cases by the number of person-years at risk given as 5-year age intervals starting at age 65 years and then multiplied by 1000 to get rates per 1000 person-years), AD rates of 2.8 per 1000 person-years in the age group 65-69 rises to 56.1 per 1000 person -years in subjects older than 90 years old. The relative increase is highest in the 75-84 year age group (Kukull, Higdon, Bowen, McCormick, Teri, et al., 2002). Interestingly, a meta-analysis of nine epidemiological studies including elderly people over the age of 80, found that the rate of increase in dementia prevalence fell in the age range 80-84. However, at around the age of 95, prevalence was seen to decrease to about 40%. This finding supports evidence for an age-related (i.e. occurring within a specific age range) rather than ageing related (i.e. caused by the ageing process itself) dementia (AD or vascular dementia), which leads researchers to consider it as a pathological process, the aetiological factors of which are in addition to those implicated in normal ageing, with consequent implications for providing therapeutic intervention (Ritchie & Kildea, 1995).

1.5.1.3 *Gender*

Research investigating the relationship between gender and AD has yielded inconsistent findings. Some of them posited that women are at higher risk of AD (Fratiglioni, Viitanen, von Strauss, Tontodonati, Herlitz, et al., 1997; Rocca, Bonaiuto, Lippi, Luciani, Turtu, et al., 1990), but others have failed to obtain a significant difference between men and women. It is interesting to note that women who carry the ApoE ε4 allele are at significantly greater risk of developing AD than men possessing the same allele (Duara, Barker, Lopez-Alberola, Loewenstein, Grau, et al., 1996).

1.5.2 Genetic

The role of several genetic factors in AD has been well established. Studies of AD among twin pairs when they were over the age of 70 provide the strongest support for genetic causation. Monozygotic twins show very high concordance rate for AD (70-80% range) (Bergem, Engedal, & Kringlen, 1997; Gatz, Pedersen, Berg, Johansson, Johansson, et al., 1997). Mutations in three genes, the amyloid precurson protein (APP) gene on chromosome 21, the presenil 1 (PS1) gene on chromosome 14 and the presenile 2 (PS2) gene on chromosome 1, characterise the familial form of AD. These forms are autosomial dominant and cause a form of the disease that usually starts in the 40s and 50s (early onset). PS1 mutations are the most frequent cause of familial early onset AD, occurring in 11% of patients (Rogaeva, Fafel, Song, Medeiros, Sato, et al., 2001). These mutations, however, account only for a small proportion (5%) of the disease. The clearest genetic factor that has been associated with "non-familial or sporadic" AD is the gene that codes for ApoE (Roses, 1996). An allelic variant of apolipoprotein E (ApoE), ε4, increases the risk of the disease fivefold in its heterozygous configuration (ε4ε3) and tenfold when both the alleles are present (homozygous configuration ε4ε4). The proportion of the disease related to ApoE ε4 has been estimated at 20% making it the single most important risk factor for this disease (Slooter, Cruts, Kalmijn, Hofman, Breteler, et al., 1998). Mutations in the genes elevate the level of the amyloid β peptide (Aβ-40 and Aβ-1-42), a preolytic fragment of the amyloid precurson protein, which subsequently aggregate in the brain in the form of neuritic plaques. The variants of the ApoE allele may be involved in the removal or degradation of amyloid β (St George-Hyslop, 2000).

In addition to the genes mainly related to familial and non-familial AD, there is a variety of other genes that might cause AD. Genetic linkage studies show additional genetic loci for AD on chromosome 12p12p and 10p24p (Bertram, Gaut, Barrett, Pinney, Whitaker, et al., 2002; Mayeux, Lee, Romas, Mayo, Santana, et al., 2002; Mayeux & Small, 2000; Scott, Grubber, Conneally, Small, Hulette, et al., 2000). Furthermore Genome-Wide Association analyses Studies (GWAS) of AD show highly significant association at the ApoE locus (Bertram, Lange, Mullin, Parkinson, Hsiao, et al., 2008a; Li, Wetten, Li, St Jean, Upmanyu, et al., 2008; Morgan, Turic, Jehu, Hamilton, Hollingworth, et al., 2007; Reiman, Webster, Myers, Hardy, Dunckley, et al., 2007). However other new susceptibility loci have been identified. The CLU gene (encodes for clustering, a major brain lipoprotein known as ApoJ), the PICALM gene (phosphatidylinositol-binding clathrin-assembly protein), and the CR1 gene [rs6656401, odd ratio (OR) =1.21)] (Harold, Abraham, Hollingworth, Sims, Gerrish, et al., 2009; Lambert, Heath, Even, Campion, Sleegers, et al., 2009). Recently a GWAS study by Mayo Clinic investigators identified a new susceptibility allele that increases the risk for late-onset Alzheimer's disease (LOAD). The gene, protocadhein 11 X-linked (PCDH11X) is located on the human X chromosome. A closely related gene, PCDH11Y, exists on the homologous region of the human Y chromosome. The study suggests that a PCDH11X variant increases the risk for LOAD, specifically in women (Carrasquillo, Zou, Pankratz, Wilcox, Ma, et al., 2009) (see Chapter 2, section 2.2.).

1.5.3 Risk modifiers

1.5.3.1 ***Alcohol***

Although excessive use of alcohol causes dementia due to nutritional deficiencies and acute toxicity, there is evidence that moderate daily amounts of wine are associated with a lower probability of developing AD in elderly individuals when compared to heavy drinkers or non-drinkers (Orgogozo, Dartigues, Lafont, Letenneur, Commenges, et al., 1997). Similar findings were obtained in a more recent study (Ruitenberg, et al., 2002). The beneficial effect of alcohol consummation might be related to its antioxidant properties or its effect on lipid metabolism (Mayeux, 2003a).

1.5.3.2 ***Mental activity***

A few studies have found that healthy people with complex activity patterns (physical and intellectual activity) have a lower risk of developing the disease than people who are sedentary and do not keep mentally active (Lindsay, Laurin, Verreault, Hebert, Helliwell, et al., 2002; Scarmeas, Levy, Tang, Manly, & Stern, 2001). However other explanations could be possible. An involuntary oversight of cause effect relationship for example, as people in the early stage of dementia, might avoid intellectual stimulation or physical activity. In this case cognitive impairment or susceptibility factors may be already present, inducing change in the behaviour of the person affected (Launer & Brock, 2004).

1.5.3.3 *Smoking*

Prospective studies have suggested higher risk of AD in heavy smokers, especially in people not carrying the ApoE ε4 mutation, through a complex interaction with disorder of the cerebral vessels (Merchant, Tang, Albert, Manly, Stern, et al., 1999; Ott, Slooter, Hofman, van Harskamp, Witteman, et al., 1998). However, an 80% reduction in Aβ peptide 1-42 deposition into plaques was observed in the brain of nicotine-treated compared to sucrose-treated AD transgenic mice (Nordberg, Hellstrom-Lindahl, Lee, Johnson, Mousavi, et al., 2002). It is important to underline that administration of nicotine through smoking does not reduce the risk.

1.5.3.4 *Down syndrome*

The risk of developing AD in people with family history of Down’s syndrome is increased threefold. Schupf et al.,(2001)found thatmothers who had a child with Down’s syndrome before the age of 35 years were at higher risk of AD than mothers who had a child with other kinds of mental retardation. The authors suggested a form of accelerated aging in the mothers of child with Down’s syndrome, predisposing them to AD.

1.5.3.5 ***Traumatic head injury***

Traumatic head injury increases the risk of AD

(Guo, Cupples, Kurz, Auerbach, Volicer, et al., 2000; Plassman, Havlik, Steffens, Helms, Newman, et al., 2000). A high percentage of AD patients report a history of head trauma, coupled with personality and cognitive changes associated with the “punch drunk syndrome” found in boxers (Lezak, 2004). The mechanism by which trauma increases the risk of AD is unknown, but there is evidence that Aβ deposition follows head injury in humans and rodents (Horsburgh, Cole, Yang, Savage, Greenberg, et al., 2000; Jellinger, Paulus, Wrocklage, & Litvan, 2001; Uryu, Laurer, McIntosh, Pratico, Martinez, et al., 2002) . Mayeux et al., (1993) suggested that the increase in risk of AD after head trauma is present only in people carrying the ApoE ε4 mutation, positing an interaction between environmental (head injury) and genetic factors in causing the phenotypic expression of the disease.

1.5.3.6 ***Cardiovascular disease***

Hyperlipidemia, hypertension, diabetes and other factors associated with stroke or heart disease have been identified as putative antecedents to AD (Breteler, 2000). The relation between cardiovascular factors and AD is stronger than middle age (Kivipelto, Helkala, Hanninen, Laakso, Hallikainen, et al., 2001; Petrovitch, White, Izmirilian, Ross, Havlik, et al., 2000). However vascular risk factors maybe strong precursor for dementia associated with cerebrovascular disease but do not appear to have an independent effect on the risk of AD (Mayeux, 2003a).

1.5.3.7 *Hormone replacement*

Women using hormone replacement therapy show a 50% reduction in disease risk (Baldereschi, Di Carlo, Lepore, Bracco, Maggi, et al., 1998; Waring, Rocca, Petersen, O'Brien, Tangalos, et al., 1999). Estrogen-deficitent animals showed amyloid peptide accumulation, and, when present, estrogen regulates the process of the amyloid precurson protein in the gamma secretase pathway (Greenfield, Leung, Cai, Kaasik, Gross, et al., 2002; Zheng, Xu, Uljon, Gross, Hardy, et al., 2002).

1.5.3.8 *Other environmental factors*

A diversity of environmental factors has also been connected with AD. Increased risk factors include exposure to metals (iron, copper, zinc, mercury and aluminium), pesticides,dietary deficiency of vitamin B or folate, and infections (Burns & Iliffe, 2009; Lahiri, Farlow, Sambamurti, Greig, Giacobini, et al., 2003; Luchsinger & Gustafson, 2009; Modrego, 2009). A decreased risk of AD has mainly been connected with dietary factors such as fruit and vegetable, antioxidants, omega-3 fatty acids, caloric and/or dietary restriction and physical activity (Scarmeas, Luchsinger, Schupf, Brickman, Cosentino, et al., 2009). Recently social factors have also been linked with the risk of AD. People living alone, being widowed or divorced in mid-life had a three times higher risk than people who were married or cohabiting (OR = 7.7) (Hakansson, Rovio, Helkala, Vilska, Winblad, et al., 2009). Illiteracy has been reported as a risk factor for AD (Kalaria, Maestre, Arizaga, Friedland, Galasko, et al., 2008). The most common speculation about how these social factors could affect the probability of developing AD seems to be related to physiological/psychological distress (Bookheimer & Burggren, 2009).

1.6 Interaction between genetic and environmental factors and the impact on AD development

There have been several studies of environmental factors that might be associated with AD. However, the findings have been inconsistent mainly because of the misunderstanding of how many variables may affect epidemiologic analyses. Investigating the relation between gene and environment might explain some of the findings. For example, a genetic factor could influence dietary preference, or level of education might be influenced by earlier genetic influences. Two studies have shown that individuals who carry one or more ApoE ε4 alleles are more likely to stop their education earlier in life (Winnock, Letenneur, Jacqmin-Gadda, Dallongeville, Amouyel, et al., 2002; Codemo, Corti, Mazzetto, Varotto, Cortella, et al., 2000). As described above, Hakansson et al., (2009) showed a significant interaction between lifestyle (living alone, being married etc) and risk of AD in old age. These authors also showed that the carriers of ApoE ε4 allele who lost their partner before mid-life and were still divorced or widowed at follow up had the highest risk of AD compared with non-carriers or with carriers who cohabited in mid-life. Finally hypertension and hypercholesterolemia, although associated with AD development, are both determined by genetic factors (Ashford & Mortimer, 2002). There are numerous other environmental factors that could be associated with AD: infection due to Herpes-simplex virus type 1 (HSV1) (Itzhaki, Lin, Shang, Wilcock, Faragher, et al., 1997) high level of Homocystein (Ho, Collins, Dhitavat, Ortiz, Ashline, et al., 2001) etc. However, such environmental factors are more likely to interact with genetic factors rather than being the main cause of AD.

Some authors have given opinions regarding the classic nature-nurture question in the genesis of AD (Ashford & Mortimer, 2002).Their position is that even the sporadic non-familial form of AD seems to be mainly due to genetic factors. This position is supported by evidence from studies of AD in twins (60% concordance), evidence of a role for environmental factors only in people with genetic predisposition, and greater increased risk of AD in first-degree relatives of people with AD etc. (Ashford & Mortimer, 2002). Although they don't dispute the possibility that certain environmental stressors may influence the age of onset of clinical AD and some environmental measures might reduce the risk of developing the disease, they support the theory that these environmental factors modify the presentation of a disease that is largely genetic in origin. However, even taking into account the very well established genetic risk factor of the ApoE ε4 genotype, the majority of AD cases are idiopathic. For this reason a multi factorial model, including neuropathological features and multiple environmental factors, has to be considered. Environmental factors may impact on either the DNA sequence itself or on epigenetic mechanisms.

Recent evidence has suggested that "gene x environment" interactions may be mediated via epigenetic mechanisms. For example epigenetic changes early in life may increase the vulnerability to cardiovascular problems such as type 2 diabetes (Gluckman, Hanson, Buklijas, Low, & Beedle, 2009) and in turn cardiovascular problems might have an association with AD (Hardy, 2009).

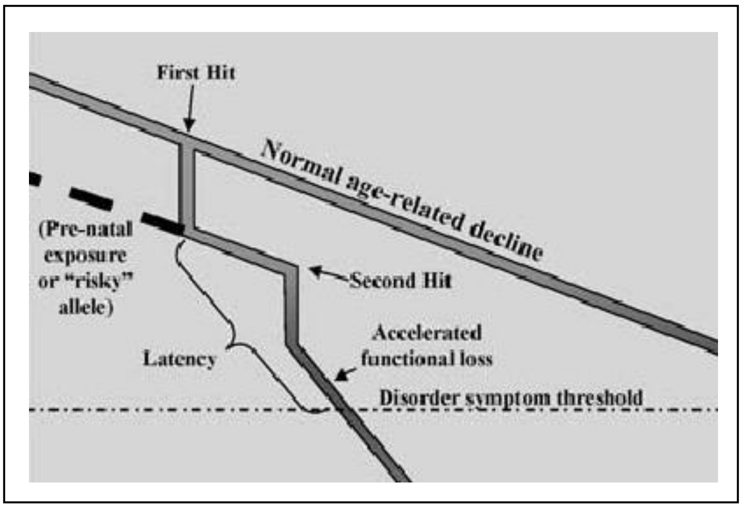
1.7 Model based theory of Alzheimer’s disease

1.7.1 LEARn model

Recently some authors have proposed the “Latent Early life Associated Regulation” model positing latent changes in expression of specific genes (Lahiri, Maloney, & Zawia, 2009). In this model environmental factors epigenetically (through DNA methilation or oxidation) disturb gene regulation in a long-term manner. The process starts in early development, but has a pathological effect only later in life. According to this model an early event (first hit) is insufficient to produce a disease state. Later in life a second hit (environmental stress) would lead to the disease only in those individuals exposed to the first hit (see Figure 1.1).

“Two-Hit” latent disease

Functionality



Age (years)

Figure 1.1. Graph plotting cognitive function across age. *An etiological agent affects an organism but does not result in a disease state. This alteration is maintained through the organism’s lifespan latently until a second hit interacts with the latent effect of the first one to produce a disease state.*

Moreover this model postulates that a gene associated with a disorder may be subjected to an environmental "primary trigger", such as epigenetic regulation, that lead to epigenetically marked genes which may undergo a temporary change in the level of expression that returns to baseline levels until a secondary trigger further affects the genes resulting in a disease state (Lahiri, et al., 2009) [Figure taken from (Lahiri, et al., 2009)].

Alzheimer's disease: A "Two-hits" disorder

For AD, the LEARn model posits a developmental triggering and latency of the APP gene until this is further triggered to a pathological level. According to this model possessing "AD associated genes" would create the first condition (hit) which triggers the initial mechanism which would then be maintained through DNA methilation latently until a second hit (environmental risk factors) would lead to the phenotypic expression of the disease. The possibility of a "two-hit" aetiology for AD has been previously recognised (Zhu, Raina, Perry, & Smith, 2004) but what the LEARn model adds to the previous hypothesis is the concept of a specific gene regulation pathway. Environmental stressors do not intentionally regulate AD-genes in the brain, but some genes are particular vulnerable to the effects of environmental factors via the alteration of DNA methilatyon and oxidation. If this were true it could be possible to posit a longitudinal model for AD and thus, individual expression profiles could be detected across the life span allowing the specific test of the effects of environmental factors in the epigenetic alterations associated with the disease. The main concept of the LEARn model is epigenome; the collection of epigenetic markers associated with a specific individual organism's genome (Whitelaw & Whitelaw, 2006). It has specific epigenotypes, generated by modification of the DNA methilation or oxidation, by change in histone acetylation patterns, or by variation of the physical arrangement of chromosomal material (van Vliet, Oates, & Whitelaw, 2007) whose expression results in sporadic neurodegenerative disorders (see Chapter 2).

Along the same line Mahley et al., (2009b) suggested a main role of the ApoE ε4 gene which by acting in concert with altered Aβ metabolism or independently might affect cognition and neuropathology (see Figure 1.2).

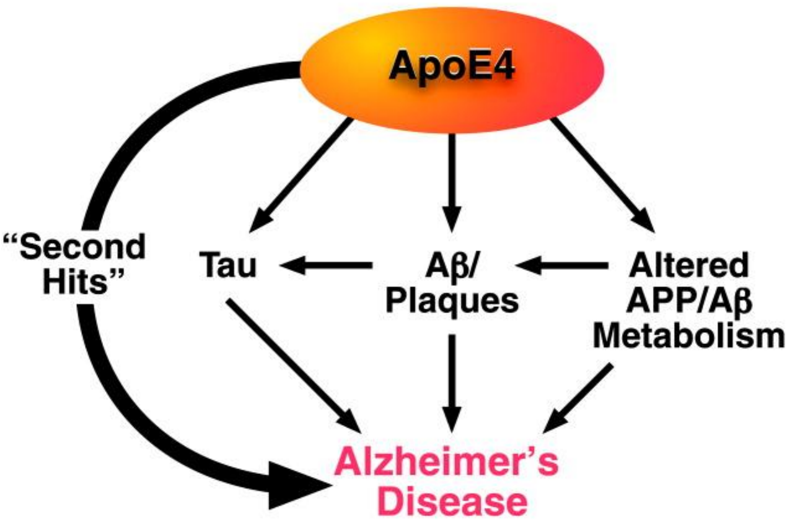


Figure 1.2. *The ApoE ε4 gene may independently and directly cause AD in response to a variety of "second hits" [Figure taken from (Mahley & Huang, 2009b)].*

According to these authors ApoE ε4 sets the stage for a "second hit" such as age, brain injury from trauma or ischemia, infection etc. These factors lead to neuronal injury and loss of synaptic connections, triggering ApoE synthesis. However, ApoE synthesis in neurons of ε4 carriers results in pathology.

Another interesting theory sharing the same background is the plasticity based theory of AD proposed by Mesulam et al., (2000). According to this theory AD promoting factors create a setting where neurons must work harder to meet neuroplasticity needs at their axonal and dendritic terminals. The appearance of neuropsychological features in AD is closely correlated with the distribution of NFTs rather than SPs. However, genetic accounts favour a disease process revolving around SPs (see paragraph 1.4.2). Advocates of perturbation of neuronal plasticity have found a common denominator to reconcile these two aspects of AD by showing evidence of a maladaptive and excessive neuroplasticity activity at the cellular level in response to initially compensatory mechanisms, as the background for the formation of NFTs and SPs (Arendt, Schindler, Bruckner, Eschrich, Bigl, et al., 1997; Furukawa, Guo, Schellenberg, & Mattson, 1998; Lanahan, Lyford, Stevenson, Worley, & Barnes, 1997; Roher, Ball, Bhave, & Wakade, 1991; Stone, Rozovsky, Morgan, Anderson, & Finch, 1998).

This compensatory pattern is triggered by the presence of AD promoting factors (such as age, low estrogen, head trauma, trisomy of chromosome 21, PS1, PS2 ApoE ε4 and AβPP mutation) which increase the burden of neuroplasticity. Over the years such compensatory processes would independently lead to chronically high levels of plasticity which leads to upregulation of tau (Brion, Octave, & Couck, 1994; Viereck, Tucker, & Matus, 1989) and AβPP turnover (Chauvet, Apert, Dumoulin, Epelbaum, & Alonso, 1997; Banati, Gehrmann, Czech, Monning, Jones, et al., 1993; Beeson, Shelton, Chan, & Gage, 1994) and thus the formation of NFTs and SPs (see Figure 1.3.). According to his theory, genetic mutations don’t really cause AD but simply accelerate the temporal course of events that lead to plasticity failure and therefore decrease the age of onset at which the pathological process begins.

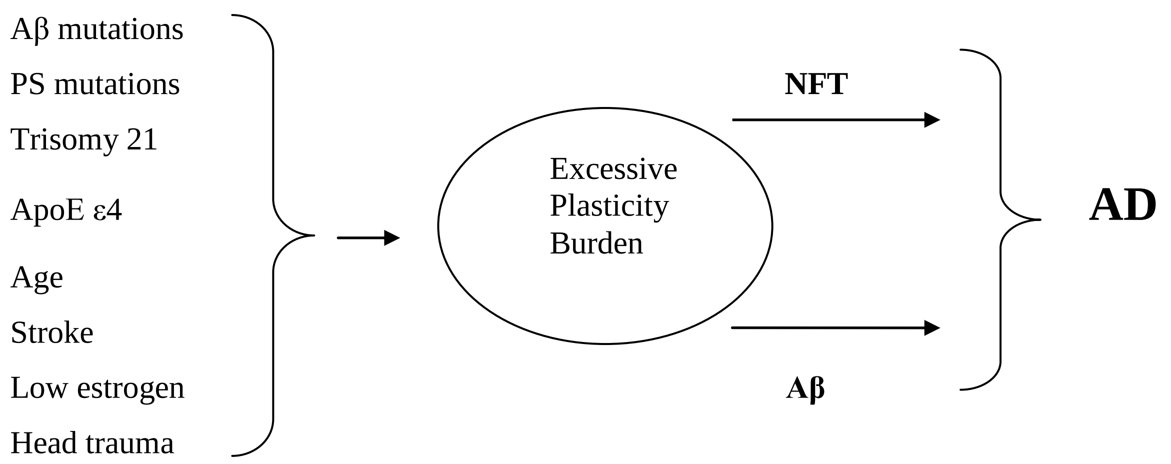


Figure 1.3. *Multiple factors increase the neuroplasticity burden that lead to plaque and NFT formation. AD is said to exist when the density of both of these histopathological markers exceeds a certain thereshold and when accompanied by dementia (Mesulam, 2000)[(Figure taken from (Mesulam, 2000]).*

CHAPTER 2 Genotype-phenotype in AD

2.1 Introduction

AD is a heterogeneous disorder in which more than 50 different genes distributed across the human genome may be involved (Cacabelos, 2002a ; Giacobini, 2000; Cacabelos, 1999a) and whose phenotypic features and current biological markers are inconsistent and do not always correlate with a defined genotypic profile (Frank, Galasko, & Hampel, 2003; Cacabelos, 2002b). Current knowledge of AD genetics doesn't explain in full the etiopathogenesis of AD, suggesting that environmental factors, cerebrovascular dysfunction and epigenetic phenomena may also contribute to AD pathology and the phenotypic expression of dementia represented by its neuropathological hallmarks (amyloid deposition in senile plaques and brain vessels, neurofibrillary tangle (NFT) formation, synaptic loss, neuronal death) and clinical symptoms (memory deficits, behavioural changes, functional decline (Cacabelos, 2002a; Giacobini, 2000; Cacabelos, 1996) (see Chapter 1 section 1.7).

2.2 Structural and functional genomics

As mentioned in Chapter 1, AD-related genes can be classified into genes with demonstrated mutations following a mendelian inheritance patterns (mutational genetics, e.g. APP, PS1, PS2), susceptibility genes or polymorphic loci potentially contributing to AD predisposition (susceptibility genetics,, e.g. ApoE, A2M, LRP1, IL1A, ACE, NOS3), and defective genes linked to mitochondrial DNA (mtDNA) (Cacabelos, 1999a, 2001, 2002a; Giacobini, 2000).

Primary loci associated with AD include the following:

- The APP gene (21q21.2-q21) encoding the amyloid precursor protein (APP).
- The presenil-1 (PS1) and the presenil-2 (PS2) genes located on chromosomes 14 (14q24.3) and 1 (1q31-q42) respectively, encoding very similar integral membrane domains whose mutations can cause familial AD3 and fAD4.
- Polymorphic variants in the ApoE gene (19q13.2) associated with risk (ApoE ε4 allele) or protection (ApoE ε2 allele) for AD.

Other genetic loci may be involved in AD in combination (or not) with environmental factors and/or epigenetic phenomena (Cacabelos, 2002a; Cacabelos, 1999a, 2002b).

Some candidate genes with polymorphic loci associated with AD and with other forms of dementia include:

- The macrotubule-associated protein tau gene (MAPT) (17q21.1) whose mutations and slicing defect can lead to fronto-temporal and familial progressive subcortical gliolisis.
- A common polymorphism (-15Ala/Thr) is the signal peptide of the α-1-antichymotrypsin (AACT) gene (14q24.3-q32.1) encoding the plasma protease inhibitor AACT. Using several lines of multiply transgenic/knockout mice, to create four AD mouse models, Nilsson et al., (2004) show that murine ApoE and human α_1-anichymotrypsin (ACT) separately and synergistically facilitate both diffuse Aβ immunoreactive and fibrillar amyloid deposition and thus also promote cognitive impairment (spatial learning ability) in aged mice. The degree of cognitive impairment is highly correlated

with ApoE and ACT-dependent hippocampal amyloid burden, with mice lacking ApoE and ACT having little amyloid and little learning disability (Nilsson, et al., 2004).

- A polymorphism in the butyrylcholinesterase (BuChE) gene (3q26.1-q26.2) is also important. Findings showed that the levels of BuChE activity and protein circulating in the CSF of AD patients were different in women and men and in carriers and non-carriers of the ApoE ε4 allele. The level of BuChE in CSF correlated positively with cognitive performance and the rate of cerebral glucose utilization. A low BuChE level in CSF may reflect a higher degree of enzyme incorporation into neuritic plaques (Darreh-Shori, Brimijoin, Kadir, Almkvist, & Nordberg, 2006).
- The low density lipoprotein-related protein (LRP1) gene (12q13.1-q13.3).
- The α-2 macroglobulin (A2M) gene (12p13.3-p12.3).
- The Type 5 AD-linked chromosome 12 gene.
- The bleomycin hydrolase (BMH) gene (17q11.1-q11.2).
- The FOS gene (14q24.3).
- The interleukin-1(IL1) gene cluster (2q14).
- The tumor necrosis factor alpha (TNF-α) gene (6q21.3).
- The β-site amyloid β-44 precursor protein-cleaving enzyme gene (BACE). (11q23.3) (BACE1, BACE2, β-secretase, memapsin-2, ASP2, p501).
- The nitric oxide synthase 3 (NOS3) gene (7q36).
- Mitochondrial DNA-associated genes.
- Other AD-related candidate genes.

None of these examined genes, however, appear to play such an important role in determining AD pathogenesis as that of the ApoE polymorphism. Nevertheless, considering that 50% of sporadic AD cases don't possess the ε4 allele of the ApoE gene, it is very plausible that many of these genes interact with each other to regulate specific metabolic pathways either confluent with or different from the amyloid cascade (Cacabelos, 2002a; Van Gassen & Annaert, 2003 ;Cacabelos, 2002b; Price & Sisodia, 1998).

From a genetic epidemiology perspective, it seems clear that the genetic dosage effect influences age of onset, as the higher the number of genes involved in AD the earlier the disease onset (Cacabelos, Mesa, & Fernandez-Nova, 1999; Mesa MD., 1999). Other genes may exert a protective effect against AD, such as ApoE ε2 (see section 2.6.3.). However, using dynamic allele specific hybridization (DASH) on polymorphic genes associated with AD (Prince, Feuk, Sawyer, Gottfries, Ricksten, et al., 2001) and other methods, previous findings of genetic associations could not be replicated (Prince, et al., 2001; Bertram, Blacker, Crystal, Mullin, Keeney, et al., 2000). Some of these contradictory results might be due to the small number of patients included in some of the studies, deficient recruitment criteria with increased sample heterogeneity and statistical dispersion of the influential genes in AD pathology.

In contrast to early-onset autosomal-dominant AD, the risk for late-onset AD is probably influenced by an array of common risk alleles distributed across different genes affecting a variety of biochemical pathways and influencing both the aetiology and pathogenesis of AD. In an attempt to identify AD susceptibility genes, a large number of studies have been carried out over the past 3 decades. With the exception of ApoE ε4, these efforts found no consistent results (Bertram, McQueen, Mullin, Blacker, & Tanzi, 2007). Recently four genome-wide association analysis studies of AD showed highly significant association at the ApoE locus (Li, et al., 2008; Bertram, et al., 2008a; Morgan, et al., 2007; Reiman, et al., 2007) , but three new susceptibility loci have been identified. The CLU gene (encodes for clustering, a major brain lipoprotein protein known as ApoJ) (rs113600, OR = 0.86), the PICALM gene (phosphatidylinositol-binding clathrin-assembly protein) (rs3851179, OR = 0.86), and the CR1 gene (Complement Receptor Type 1) (rs6656401, OR =1.21) (Harold, et al., 2009; Lambert, et al., 2009). CLU and PICALM were shown to be protective; CLU seems to be involved in Aβ clereance. Moreover, in the area of epigenetic-relevant genes, the C677T polymorphism in MTHFR has been significantly associated with AD, (OR= 1.13) (Bertram, Hsiao, McQueen, Parkinson, Mullin, et al., 2007). Finally, another recent GWAS study identified a Single-Nucleotide Polimorphism (SNP) (rs5984894) on the human X chromosome (Xq21.3), in the protocadhein 11 x-linked (PCDH11X) that is strongly associated with LOAD in individuals of European descent from the United States, specifically in women. Of the SNPs that demonstrated genomewise significance, 6 were linked to ApoE. rs5984894 was the only SNP not linked to ApoE. Analysis of rs5984894 by multivariable logistic regression adjusted for sex was performed. Odds ratios were 1.75 for female homozygotes [p = 2.0 x 10(-7)] and 1.26 for female heterozygotes (p = 0.01) compared to female non-carriers. For male homozygotes (p = 0.07) compared to male non-carriers, the odds ratio was 1.18. PCDH11X encodes protocadhein 11, one of the families of cell surface receptors involved in cell-cell adhesion, process important for the neuronal development and formation of functional synapses. Differential expression of the PCDH11X gene in individual neurons may alter cell adhesion. Interestingly, presenil 1, a protein important in processing of beta-amyloid in AD, forms complex with neuronal-cadherin. Alteration in this interaction may change the final result. Because the epsilon 4 allele of the ApoE is known to reduce the brains ability to rid itself of amyloid beta and also PS1, PS2 and AAP both influence the amyloid beta protein, it could be that this rs5984894 also influences the amyloid beta protein (Carrasquillo, et al., 2009).

2.3 Epigenetic regulation

Many neurological and psychiatric disorders are not due to mutations of a single gene, rather they involve molecular disturbances entailing multiple genes and signals that control their expression. They share a substantial genetic predisposition and a contribution of environmental factors. Recent research has raised the issue that epigenetic mechanisms, which exert lasting control over gene expression without altering the genetic code, could mediate stable changes in brain function. The influence of epigenetic phenomena in neurodegeneration is practically unknown and most environmental factors of risk of dementia are not well characterised.

However several characteristics of AD are compatible with an epigenetic component. For example, the discordance in monozygotic (MZ) twin pairs indicates that non-genetic factors such as environmental and epigenetic factors could play a significant role (Brickell, Leverenz, Steinbart, Rumbaugh, Schellenberg, et al., 2007; Petronis, 2006). Fraga et al., (2005) showed how MZ twins display an epigenetic “code” similarity proportionally to the time they have spent together and to their age. Recently some studies have shown how environmental factors can affect epigenetically a person’s phenotype, showing how some environmental exposure can induce epigenetic changes in a diversity of tissue sample (Liu, 2008).

The idea that the phenotype arises from genotype through programmed change, is the original definition of epigenetics by Waddington in 1942 (Van Speybroeck, 2002) which mainly overlaps with the modern definition of epigenetics information which is heritable during cell division other than from the DNA itself. These two definitions share basically the same concept of epigenetic regulation on developmental processes; different cell types maintain their fate during cell division even though their DNA sequences are essentially the same.

What is an epigenetic disorder? Several defects in the epigenome are known to lead to disease, including change in the localised or global density of DNA methilation and incorrect histone modification. Studies of epigenetic mechanisms that underlie heritable transmission have flourished in the fields of developmental and cancer biology, where the continuity of unique patterns of gene expression between parent and daughter cell is crucial. These studies have converged on a set of common enzymatic modifications to the chromatin structure that can up or down regulate gene expression in a manner that is transmissible to daughter cells. These mechanisms also regulate gene expression in neurons but, as most neurons do not divide, chromatin modifications are instead sustained within individual cells (Tsankova, Renthal, Kumar, & Nestler, 2007). Fundamental neurodevelopmental processes, such as cell fate specification and neurogenesis, are highly regulated at the level of chromatin remodelling. One of the best-established examples is the transcription Neuron-Restrictive Silencing Factor (NRSF). It represses neural differentiation by binding to conserved NRS Elements (NRSEs) in gene prompters in non-neuronal cells. More recently, NRSF has been shown to modulate the expression of NRSE-containing gene in mature neurons; inhibition of NRSF leads to neuronal activation and the promotion of neurogenesis (Kuwabara, Hsieh, Nakashima, Taira, & Gage, 2004). Chromatin remodelling may also be involved in the regulation of adult neurogenesis, which occurs in a highly restricted brain region: the subgranular zone of the hippocampus dentate gyrus and the subventricular zone adjacent to the striatum. Although chromatin remodelling is best understood for its influence in neural development, increasing evidence suggests a role in regulating mature, fully differentiated neurons. During synaptic transmission, neurons respond to neurotransmitters by receptor-mediated intracellular signal transduction events that, among other actions, activate or inhibit transcription factors. The regulation of transcriptional activity by transcription factors binding to DNA depends on the interactions of the transcription factors with many co-activators or co-repressor and the underlying structure of chromatin. Chromatin remodelling is thus intimately linked to activation or repression of genes by synaptic activity and thus the regulation of complex behaviour (Hsieh & Gage, 2005).

2.3.1 Overview of epigenetic mechanism

Chromatin is the complex of DNA, histones and non-histones protein in the cell nucleus. The fundamental unit of chromatin is the nucleosome, which consists of about 147 base pairs of DNA wrapped around a core histone octamer. Each octamer contains two copies of the histones H2A, H2B, H3 and H4 (see Figure 2.4a). The nucleosomal structure of chromatin allows the DNA to be packaged into the nucleus by organized folding (Felsenfeld & Groudine, 2003). Chromatin exists in an activated, condensed state called heterocromatine, which does not allow the transcription of genes, and in an active open state called eurochromatine, which allows individual genes to be transcribed. In reality chromatine can exist in many states in between these two extremes, some portion can be repressed or be in a permissive state. The genes, however, are available for derepression or activation in response to transcription factors and transcriptional co-activators (see Figure 2.4b). Moreover, this change in gene state happens in a high temporal and spatial resolution by permitting small groups of nucleosomes to become more or less open allowing the transcriptional process in specific regions or not.

Experiments have yielded detailed information about the molecular mechanisms that control chromatin architecture in order to alter gene expression. Several general mechanisms have emerged and it is generally believed that their complex interactions determine the appropriate expression of specific genes in eukaryotic cells (Choi & Friso, 2005; Hake, Xiao, & Allis, 2004; Felsenfeld & Groudine, 2003) (see Figure 2.4). Changes in chromatin structure are related to epigenetic modifications that consist of DNA methylation, histone post-trascriptional modifications (methylation, acethylation and phosphorylation) and ATP-mediated chromatin modifications. In proliferating cells, the DNA is principally found as euchromatin, in actively transcribed loci like the growth regulatory genes. Conversely, it has been proposed that reassembly of repressive chromatin domains (heterochromatin) may contribute to cellular senescence.

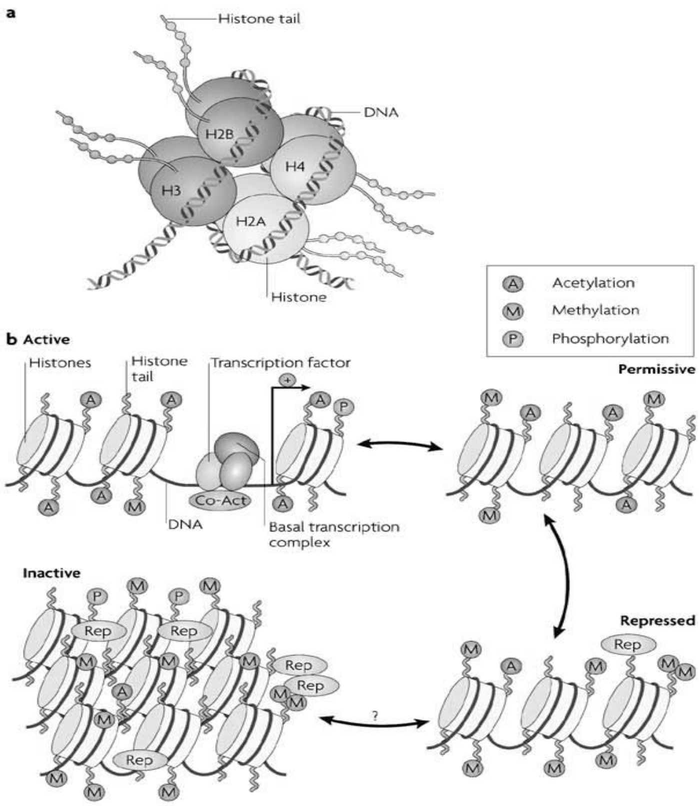


Figure 2.4. *General scheme of chromatin remodelling [Figure taken from (Tsankova, et al., 2007)].*

The main tool of epigenetic control in gene expression seems to lie in the CpG sequence and whether or not this sequence may contain a methyl group bound on C (Razin & Riggs, 1980). Although most studies have been dedicated to DNA, methylation processes are also involved with RNA, proteins and lipids (Chiang, Gordon, Tal, Zeng, Doctor, et al., 1996). Gene silencing through DNA methylation is only the remethylation of the cytosines of CpG sequences eventually present in the gene promoters of those genes whose regulation is exerted by the methyl group (Razin, 1998).

There is a basic difference between sequences that are normally unmethylated, like CpG islands (Bergman & Mostoslavsky, 1998; Siu, Chan, Wong, Choy, & Kwong, 2003; Cross, Meehan, Nan, & Bird, 1997), and the CpG moieties belonging to genes expressed during development that are silenced later by methylation for physiologic reasons. CpG islands may become methylated for a pathogenetic mechanism. For example, in the inactivation of oncosoppressor genes in cancer, CpG moieties may gradually lose their methylation and therefore overexpress genes that should be down regulated. A similar mechanism seems to be involved in the outcome of AD.

2.3.2 DNA-methylation

DNA methylation is another important mechanism of gene regression. The functional significance of DNA methylation is best established in X chromosome inactivation (such as Fragile X syndrome) and genetic imprinting (such as Angelman syndrome). More recently, DNA methylation has been implicated in the regulation of gene activity in the adult brain either in normal or pathological conditions.

Such mechanisms regulate the expression of specific sets of neural genes that are important for neural activity, survival, morphology and ultimately the integrated regulation of complex behaviour. The study of DNA methylation in aging is extremely topical because of its implication in tumorigenesis, since cancer onset increases with aging (Neumeister, Albanese, Balent, Greally, & Pestell, 2002). During embryonic development, DNA undergoes the establishment of the inherited methylation pattern of the adult organism with the formation of stably activated genes (mostly demethylated), stably silenced genes (mostly fully methylated) and of genes with specific methylation patterns able to be induced by reassessment of methyl moieties. With aging of the organism, the general DNA demethylation can lead to reactivation of stably silenced genes or to over-actiavtion of methylation-inducible genes (see Figure 2.5). The mechanisms that regulate global hypomethylation of DNA with aging and the concomitant increase in *de novo* methylation at specific sequences remain to be better clarified. Several DNA-methyltransferases (DNMTs) exist. Maintenance methylation of hemimethylated DNA during replication is guaranteed by DNMT1, whereas the sequences-specific increase in *de novo* methylation depends on the increased activity of the other DNMTs (Liu, Wylie, Andrews, & Tollefsbol, 2003). Alterations in DNA methylation during aging can depend on alterations in dietary status and it is largely accepted that nutritional components have great influence on health and lifespan. Among the various mechanisms by which nutritional elements could affect the progress of senescence, two pathways involve DNA methylation; the first concerns the supply of metabolities of S-adenosylmethionine cycle (SAM, folic acid and B vitamins), whereas the second refers to elements able to directly modify DNMT activity (selenium, cadmium and nickel). In conclusion, methylation patterns established during development are not definitive in adulthood; however there is a growing body of evidence linking epigenetic alterations to the development of neurologic disorders. Aberrant DNA methylation and histone modification mechanisms caused by a mutation in certain genes (i.e., epigenetic gene), can cause several neurodevolpmental disorders and are involved in neurodegenerative diseases and other neurological pathologies. Clear indications of methylation alterations come from studies on Prader-Willi, Angelman's and Beckwith-Wiedemann syndromes (Kaufman, Heled, Perk, Razin, & Shemer, 2009; Manipalviratn, DeCherney, & Segars, 2009) and on various diseases related to ageing (AD, Parkinson's and Huntington's diseases) (Maeda, Guan, Oyama, Higuchi, & Makino, 2009; Urdinguio, Sanchez-Mut, & Esteller, 2009).

Given these considerations, an epigenetic approach seems necessary to understand the mechanisms that regulate ageing and its related disease; hopefully providing a better molecular tool for improved diagnosis, prognosis and therapy of these pathologies.

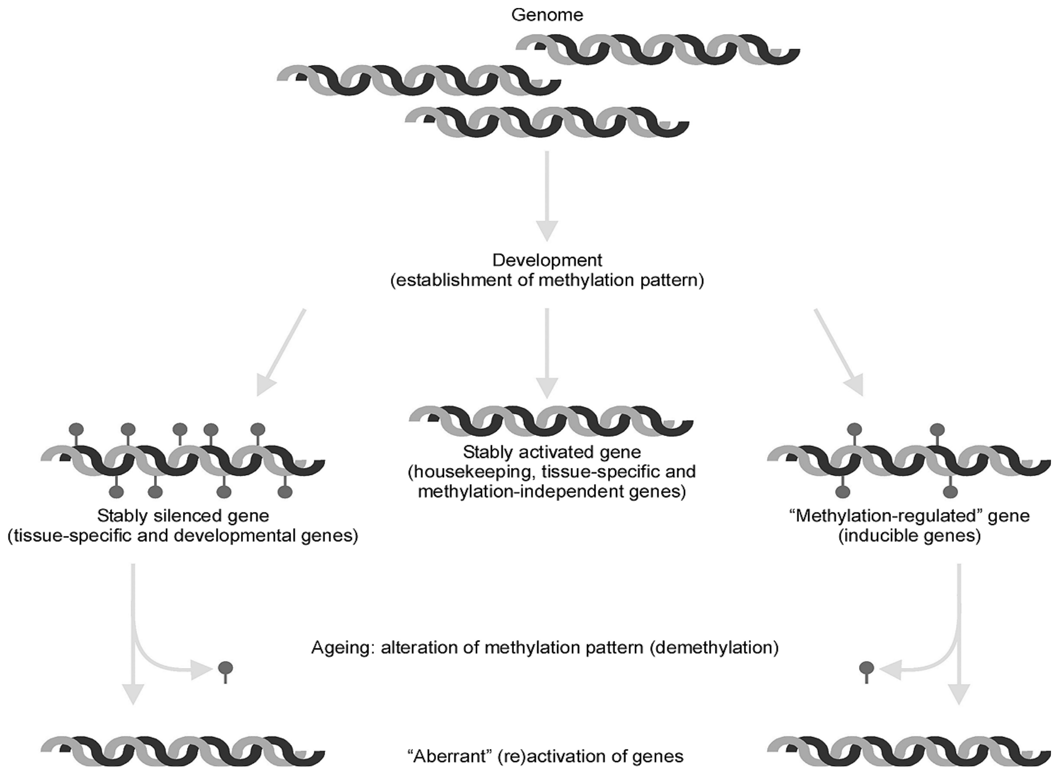


Figure 2.5. *DNA methilation in development and ageing [Figure taken from (Scarpa, Cavallaro, D'Anselmi, & Fuso, 2006)].*

2.3.3 Methylation and Alzheimer's disease

A recent study underlines that AD is among the few diseases that may display high homocysteine (HCY) and low B12, B6 and folates in the blood (Scarpa, et al., 2006). This observation has raised the question of whether amyloid-β overproduction and accumulation, which may be implicated in the genesis of the disease, could be due to the loss of epigenetic control in the expression of the genes involved in AβPP (amyloid-β protein precursor) processing. The authors showed that, in cell culture, two of the genes responsible for amyloid-β production are controlled by methylation of their promoters. The process is strictly related to S-adenosylmethionine (SAM) metabolism. SAM is a natural compound, mainly produced by the liver. It is considered the primary methyl donor present in all living organisms involved in the methylation of target molecules such as DNA, proteins, lipids and polyamines synthesis (Fontecave, Atta, & Mulliez, 2004; Razin, Szyf, Kafri, Roll, Giloh, et al., 1986). SAM is probably second only to ATP in the variety of reactions in which is involved. Homocysteine is derived from the demethylation of S-adenosylhomocysteine (SAH) and further hydrolysed into homocysteine and adenosine. The remethylation of homocysteine to form methionine, by the remethylation pathway, prevents its accumulation.

Several authors have described what kind of damage can be generated by the accumulation of HCY and DNA appears to be one of the most important targets (Chiang, et al., 1996). However lack of SAM production and SAH accumulation seem to be equally important in causing damage (Scarpa, et al., 2006). Active demethylation without DNA replication has been shown to be possible and probably frequent in tissue repair mechanism (Jost & Jost, 1995). Aside of the physiological function of these epigenetic phenomena, they may be influenced by metabolic alterations. The alteration of metabolities and of the enzymes part of the methyl donor (SAM) metabolic cycle may be responsible for its reduced production. The consequent demethylation and overexpression of genes would not be regulated but rather induced by the reduced synthesis of the methyl-donor or by its inhibition. It could be the case of HCY accumulation due to decreased B12, B6 and folate uptake. HCY, if not rapidly transformed in methionine, from SAH, a potent inhibitor of methyl-transfer reactions. These metabolic alterations may also be responsible for the generalised reduction of DNA methylations observed in ageing. In AD, the loss of a precise control through gene methylation may alter a delicate equilibrium among the three enzymes (alpha, beta and gamma secretases) known to be involved in the production of amyloid-β (De Stropper, 2000).

It has been demonstrated that the two genes responsible for amyloid-β production, beta and gamma are regulated by methylations in their promoters (Scarpa, Fuso, D'Anselmi, & Cavallaro, 2003). Moreover, authors have shown that the feeding of neuroblastoma cells with a culture medium deprived of B12 and folates increased amyloid-β production (Fuso, Seminara, Cavallaro, D'Anselmi, & Scarpa, 2005). The administration of methyl-donor had the opposite effect; remethylating the genes reduced significantly amyloid β levels. As for the HCY/SAM dismetabolism, the most critical point is the alteration of the SAM/SAH ratio, also known as methylation potential, rather than the increase of homocysteine concentration that may produce biological damage (Ulrey, Liu, Andrews, & Tollefsbol, 2005). The alteration of methylation patterns could be produced either by lack of methyl-donor or by methyl-transferases inhibition. SAM synthesis could be lowered by diminished vitamin uptake, B12 and folates, as well as by inhibition of the enzymes (MAT) involved in the transformation of methionine to S-adenosylmethionine. Inhibition of methyl-transferases instead is more likely bound to accumulation of Homocysteine and SAH. Whether or not these are the primary causes of AD, they may contribute to its development. Prevention might pass through the control of methylation metabolism, by measuring SAM/SAH ratio and HCY, folates and B12 levels in blood. Further support for this thesis comes from the observation that in the elderly, DNA methylations are consistently lower than in young and middle aged people. For example, Mastroeni et al., (2008) showed DNA hypomethylation in the enthorhinal cortex of AD when compared to controls. Similarly, in the temporal neocortex of monozygotic twin pairs discordant for AD there was evidence of different DNA methylation (Mastroeni, McKee, Grover, Rogers, & Coleman, 2009). A recent study using genome-wide analysis of DNA extracted from prefrontal cortex showed a distinct differential pattern of DNA methylation between AD and controls (Zukin, 2009). Finally, Wang et al., (2008) found that the epigenetic distance from the norm (the median methylation of healthy control individuals) was higher in brains of people with AD than in healthy controls, and that this "epigenetical distance" increases with the age.

2.4 Genotype-phenotype correlation

A practical approach to understanding the potential influence of a particular gene or a genomic cluster on a specific phenotype is to study genotype-phenotype correlations. The phenotype is the sum of observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences. Genetic influences account for over 50% of the variance in adult cognitive abilities, and arise from contributions to the life-long trait of intelligence and to any influences specific to old age (Plomin & Spinath, 2002). Each genetic influence is referred to as a quantitative trait locus (QTL), but in the absence of robust QTL mapping information on cognitive ageing trajectories, the choice of candidate genes is frequently unreliable. Due to the low efficiency of candidate-gene selection, large number of negative association studies have been reported and fewer than half of QTL associations have been replicated (Deary, Wright, Harris, Whalley, & Starr, 2004). However, a well validated approach used combination of mapping or positional information and candidate gene selection within a limited region of the genome (Linkage approach) ("The Human Gene Mutation Database.," 2008). To obtain positional information, a gene mapping in families or in groups of people sharing the same trait of interest is needed. An extension of this method for QTL mapping is to carry out whole-genome association scans on individuals of known trait value using very large number of single nucleotide polimorphisms (SNP) markers. The trait value is correlated with the presence of nearby genetic markers. In this regard the effect of the ApoE on cognitive decline was first identified in families with late onset Alzheimer's disease (Deary, et al., 2004).

The ApoE ε4 is said to be a "frailty gene, predisposing one to be more susceptible to injury and less likely to recover from trauma once it occurs" (Smith, 2002) (p. 355-356). People who are carriers for the ε4 allele of the ApoE gene are more likely to suffer early death, cardiovascular disease, stroke, and Alzheimer's dementia (Farrer, et al., 1997; Smith, Andersen, Kryscio, Schmitt, Kindy, et al., 2002). A genetic profile which includes one ApoE ε4 allele is associated with lower cognitive performance in non-demented older people (Anstey & Christensen, 2000; Pendleton, Payton, van den Boogerd, Holland, Diggle, et al., 2002). Moreover this genetic variant benefits by having a relatively well understood mechanism (Smith, et al., 2002; Mahley & Rall, 2000). Unlike the genes involved in the familial variant of AD (APP, PSE1, PSE2), ApoE ε4 is a better established 'genetic-modifier' of cognitive function. This stands out as an unusually robust QTL finding in human psychology, encouraging further steps to be made. After finding an association between genetic variability and phenotype differences, comes the search for a mechanism.

2.5 Endophenotype

An important step toward the understanding of the underling mechanisms associated with AD is to identify genes which influence brain structure under specific physiologic circumstances. Data have showed thatlate-onset Alzheimer’s disease LOAD is characterised by functional and structural brain changes and that geneticconditions affect these modifications (Thompson, Cannon, & Toga, 2002; Thompson, Cannon, Narr, van Erp, Poutanen, et al., 2001). Genetics appears to influence brain modification along the entire human lifespan from childhood to old age (Thompson, Hayashi, Dutton, Chiang, Leow, et al., 2007), with the pathologic phenotype representing only the last stage of this influence. In this sense, these data are useful not only to understand better the relation between genetic variability and neuroanatomy in general, but also to clarify the specific role of some genes in this neurodegenerative disorder in terms of structural brain changes and cognitive reserve (CR), a model on reserve against brain damage created to explain the indirect relationship between the severity of the AD and the degree of cognitive impairment (Reitz & Mayeux, 2009).The concept of CR comes from the observation of a different level of neuropathological and clinical involvement in LOAD. Favourable factors such as high education level or genetic predisposition help people to develop CR and in turn make them bear greater brain damage before cognitive deficits appear (Stern, 2002). This CR concept can be applied to any situation where there is variability in brain injury response, suggesting that CR can be applied to healthy individuals but also to people with neurodegeneration. In this regard a conjunction analysis of genetic and neuroanatomic factors involved in CR process could help to clarify the underling mechanisms leading to the clinical manifestation of LOAD.

To evaluate putative genetic and non-genetic modifiers of the risk for neurological disorders, it would be helpful to identify an "endophenotype". Gottesman (2003) defined it as *"measurable components unseen by the unaided eye along the pathway between disease and distal genotype"* **(**p. 636**).** That is, a measurable intermediate phenotype, that is generally closer to the action of the gene than disease status and thus exhibits a higher genetic signal-to-noise ratio (Gottesman & Gould, 2003). For example, a substantial proportion of the genetic influence on differences in brain volume and structure is shared with differences in cognitive ability (Carmelli, Swan, DeCarli, & Reed, 2002 ; Thompson, et al., 2001). There are characteristics that are genetically correlated and can be measured in both affected and unaffected individuals. Moreover, endophenotypes often provide much greater power to localise and identify disease-related QTLs than disease status alone does, providing more informational phenotypes (Blangero, Williams, & Almasy, 2003) (see Figure 2.6). Among the most used endophenotypes, plasma amyloid β level, a putative risk factor, has not been used as often as age of onset and cognitive test performance in genetic studies of LOAD (Farris, Leissring, Hemming, Chang, & Selkoe, 2005). Genetic risk factors in LOAD studies which are associated with age of onset and cognitive performance show that ApoE ε4 is the most common gene risk factor for LOAD, and tends to lower the age of disease onset (Blennow, de Leon, & Zetterberg, 2006). Some authors have attempted to correlate the presence of the ApoE ε4 allele with phenotypic traits represented by pathogenic hallmarks and clinical features of dementia with very variable results (Cacabelos, 2001; Cacabelos, 1999a;

Saunders, Trowers, Shimkets, Blakemore, Crowther, et al., 2000). Findings have previously demonstrated that the ApoE ε4 genotype influences the phenotypic expression of different clinical symptoms (cognitive decline, behavioural changes and functional disability), biological parameters (brain atrophy, lymphocyte apoptosis, serum ApoE and beta-amyloid protein levels) and therapeutic responses in AD (Cacabelos, 2002a; Cacabelos, Fernandez-Novoa, Lombardi, & Takeda, 2003a; Cacabelos, 2002b; Cacabelos, 2001; Cacabelos, 1999a; Cacabelos, Alvarez, Fenandez-Novoa, & Lombardi, 2000). In this regard it is worth investigating this protein more extensively and the underlying mechanisms associated with this increased risk.

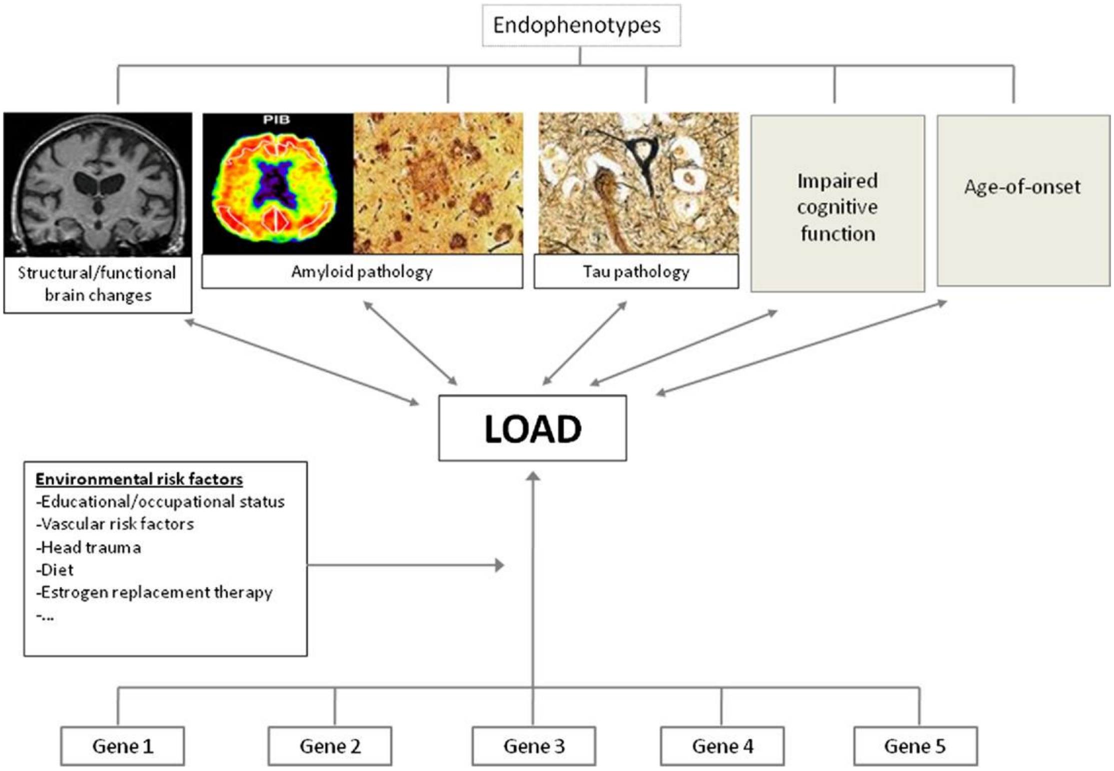


Figure 2.6. *Endophenotypes in the cascade between variance in a genetic sequence and the AD syndrome [Figure taken from (Reitz & Mayeux, 2009)].*

2.6 Apolipoprotein Epsylon 4

2.6.1 Historical background

The clearest genetic factor that has been associated with non-familial or sporadic AD is the gene that codes for ApoE (Plassman, et al., 2000). This gene has been identified as a major factor in causation of AD in cases that occur predominantly over 60 years of age and do not have an apparent autosomial mode of inheritance. The epsilon 4 mutation of the ApoE gene is the most consistently replicated gene involvement in sporadic AD with an OR of 3.6 (Bertram, Hsiao, et al., 2007).

Discovered in the 1970s, this 34-kDa, 299-amino acid (aa) protein was identified in triglyceride-rich lipoproteins and induced by cholesterol feeding in animals and humans (Mahley, 1983, 1988; Mahley & Rall, 2000) . The amino terminal domain (1-191) is a stable globular structure containing a receptor binding site, while the carboxy-terminal domain (residues 216-299) is helical, less stable, and contains the lipoprotein binding functions (Weisgraber, 1994). A polymorphism of ApoE in the human serum was first described by Utermann et al., (1979). The ApoE gene was firstly localised on chromosome 19 because of its linkage with a locus (C3 complement component) (Olaisen, Teisberg, & Gedde-Dahl, 1982). Genetic mapping was then refined and ApoE assigned to 19q, while the C3 locus was localised in the ApoE arm of the chromosome (see Figure 2.7.) (Lusis, Heinzmann, Sparkes, Scott, Knott, et al., 1986).

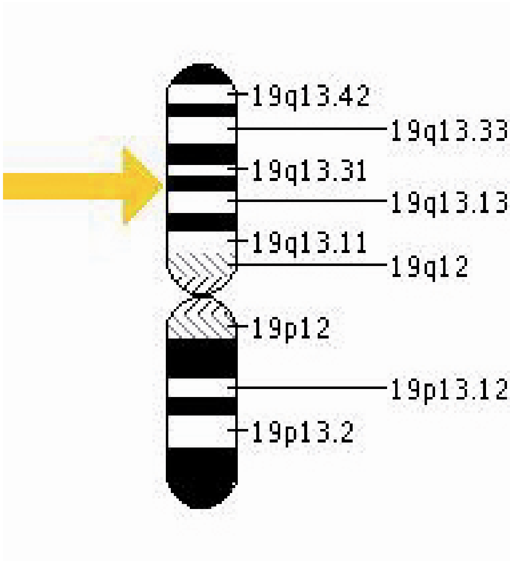


Figure 2.7. *ApoE localization on chromosome 19 [(Figure taken from http://wiki.medpedia.com / Apolipoprotein_E_(APOE)]*

Zennis et al., (Zannis, Just, & Breslow, 1981) identified by isolectric focusing the three major isoforms of ApoE (ApoE ε2, ApoE ε3, ApoE ε4) and concluded that a single locus with three alleles (ε2, ε3 and ε4) is responsible for this pattern. The ApoE ε2, ApoE ε3 and ApoE ε4 isoforms differ in amino acid sequence at two sites, residue 112 and residue 158:

E2: NH2----Cys112----Cys158----COOH
E3: NH2----Cy112-----Arg158----COOH
E4: NH2---Arg112----Arg158----COOH

The three alleles differ in their frequencies: ε4 (15-20%) ε3 (65-70%) and ε2 (5-10%) and give rise to three homozygous and three heterozygous phenotypes. The nomenclature arose by consensus among key investigators (Zannis, Breslow, Utermann, Mahley, Weisgraber, et al., 1982). Corbo and Scacchi (Corbo & Scacchi, 1999) analysed the ApoE allele distribution in a variety of populations and found that the ε3 allele is the most frequent in all human groups (Payami, Zareparsi, Montee, Sexton, Kaye, et al., 1996). Interestingly, projected number estimations of AD in the U.S.A. based on the number of AD cases and frequency of the ApoE genotype in patients and controls from multi-site studies, show that there would be about half the number of AD cases in the U.S.A. if the ApoE ε4 allele did not exist. The ε4 allele by itself is, therefore, responsible for about 50% of the non–familial AD cases in that country. The two percent of the population with the ε4ε4 genotype has a 15 times higher risk than the 60% of the population that has ε3ε3 genotype. By the age of 80 years, 91.3% of patients with the ε4ε4 genotype, 47.8% of ε3ε4 individuals and only 20% of those without an ε4 allele have AD (Bagnoli, Nacmias, Tedde, Guarnieri, Cellini, et al., 2002; Crawford, Freeman, Schinka, Abdullah, Richards, et al., 2000; Citron, Oltersdorf, Haass, McConlogue, Hung, et al., 1992; Corder, Saunders, Strittmatter, Schmechel, Gaskell, et al., 1993; Bowen, Allen, Benton, Goodhardt, Haan, et al., 1983) . Moreover, the ApoE genotype has an effect on age related prevalence of AD, with the ApoE ε4ε4 individuals having as estimated 50% chance of AD onset at 68.4 years old, the ApoE ε3ε4 individuals at 75.5 years, and the ApoE ε3ε3 individuals at 84.3 years (Arendt, et al., 1997). The ApoE ε4 allele confers its maximal effect on risk before the age of 70 (Alvarez-Arcaya, Combarros, Llorca, Sanchez-Guerra, Berciano, et al., 2001), partly explaining why studies looking at the effect of this gene in older populations have not found a significant effect of this allele.

2.6.2 Functional role of ApoE

ApoE is expressed in several organs. The highest expression of plasma ApoE (~40-70 μg/ml) is in the liver (>75%) followed by the brain (Elshourbagy, Liao, Mahley, & Taylor, 1985). Although non-neuronal cells, mainly astrocytes, are the major cell types that produce a large proportion of cerebrospinal fluid ApoE (~3 to 5 μg/ml) (Mahley, 1988; Elshourbagy, et al., 1985; Mahley, 1983), neurons synthesize ApoE when stressed (Xu, Bernardo, Walker, Kanegawa, Mahley, et al., 2006). ApoE functions as a ligand in receptor-mediated endocytosis of lipoprotein particles (triglyceride- and cholesterol-rich lipoproteins). In plasma, the ApoE protein is present on lipoprotein in association with other apolipoproteins, whereas in the brain ApoE and two other apolipoproteins ApoJ and ApoA-1, are present on high-density-like lipoprotein particles (Fagan, Holtzman, Munson, Mathur, Schneider, et al., 1999; Pitas, Boyles, Lee, Foss, & Mahley, 1987). The major component in the plasma is ApoA-1 whereas in the Central Nervous System (CNS) there is a predominance of ApoE (Pitas, et al., 1987). Initially, ApoE was shown to be involved in lipid transport and cardiovascular disease (Mahley, 1983; Mahley & Rall, 2000). After low-density lipoprotein receptor (LDL) was identified (Goldstein & Brown, 1976), ApoE has been identified as a major ligand (Mahley & Rall, 2000; Mahley, 1988; Innerarity & Mahley, 1978; Innerarity, Pitas, & Mahley, 1979). It is also a ligand for other members of the LDL receptor family, including the LDL receptor-related protein, which is involved in lipoprotein clearance. Moreover, ApoE also binds to heparin sulphate proteoglycans (HSPG), the study of which has shown that ApoE has a role in artherosclerosis (Mahley & Rall, 2000; Mahley, Huang, & Rall, 1999), modulates susceptibility to infectious disease and immunoregulation (Mahley, 1983; Mahley & Rall, 2000), enhances the infectivity of HIV in vitro and accelerates progression to

AIDS and death in HIV-positive subjects (Burt, Agan, Marconi, He, Kulkarni, et al., 2008). ApoE also plays a key role in the neurobiology of AD (Mahley, Weisgraber, & Huang, 2006).

After the discovery that there is immunoreactivity of ApoE in amyloid plaques (Wisniewski & Frangione, 1992; Namba, Tomonaga, Kawasaki, Otomo, & Ikeda, 1991), the ε4 allele was discovered as a major risk factor for AD, with 60-80% of the AD population having at least one ε4 allele (Corder, et al., 1993; Strittmatter, Saunders, Schmechel, Pericak-Vance, Enghild, et al., 1993), with a 12 fold increased risk for the homozygous type (ε4ε4) (Bertram, 2009). Finally, analysis of the ApoE structural domains provided insight into the mechanism by which ApoE has a role in cardiovascular, neurological and infectious diseases (Mahley, 1983; Mahley, Weisgraber, & Huang, 2009a). Although the three common isoforms differ by only one or two aminoacids at the residue 112 or 158, these amino acid differences profoundly alter the structure and function of ApoE (Mahley, et al., 2009a).

2.6.3 Evolutionary Theory as an explanation of a nurture-nature interaction

As summarised above, a number of studies showed that the ApoE ε4 allele is involved in a variety of pathologies (cardiovascular, neurological and infection diseases). Furthermore, some authors have suggested that it might have a role in acting as a first necessary, but not sufficient, factor in triggering neurodegenerative diseases. Environmental factors have to be added in order to reveal the phenotypic expression of the pathology (see section 1.7. Chapter 1).

To understand the fundamental role of genetic factors in an environmental context, an evolutionary perspective should be taken in account. According to this point of view, the physiology of an organism, after the end of the reproductive period, could be the manifestation of epigenetic events occurring on the basis of genetic development from previous stages of life. Now the evolutionary pathway of the ApoE ε4 allele is clear. It seems that this allele was the ancestral gene until 300,000 years ago when the ε3 allele appeared, followed by the ε2 allele 200,000 years ago (Fullerton, Clark, Weiss, Nickerson, Taylor, et al., 2000). Although the exact cause of the additional alleles (ε3 and ε2 allele) is unknown, some authors have suggested that they might have some beneficial effects in agrarian societies, especially in those with greater longevity (Corbo & Scacchi, 1999), maybe giving them superior cognitive and cardiovascular skills in order to guarantee more protection in a more organised social environment. In this regard, patriarchs could control their tribes more ably and keep procreating and matriarchs could take care of the healthier development of their progeny. In this contest, factors promoting brain development and neuroplasticity, like cholesterol, play a key role in building up new synapses. ApoE mediates the role of cholesterol (Mauch, Nagler, Schumacher, Goritz, Muller, et al., 2001; Poirier, 2000) which has been seen to have an important role in AD and the metabolism of β-amyloid (Fassbender, Simons, Bergmann, Stroick, Lutjohann, et al., 2001; Snowdon, et al., 1996). As the human life span increased, greater pressure on cerebral neurons to store information could have started in an attempt to cope with the numerous physiological stressors associated with aging, including active oxygen species (ROS) (Ashford & Mortimer, 2002). This hypothesis could support neuroplasticity based theory of AD positing that the formation of NFTs and SPs might be independent consequences of excessive plasticity-related cellular activity and the loss of neurons, dendrites and synapses would then be the ultimate expression of plasticity failure (Mesulam, 2000). In this regard, improved cholesterol management could have been the key development offered by the ApoE ε3 and ε2 allele. Traces of this ApoE-environment relationship are still evident in modern times, by observing the different ApoE frequencies across various populations. ApoE ε4 allele is most common in the African pygmies (41%), least common in Sardinians (5%) and intermediate in most Western populations (9-19%) (Corbo & Scacchi, 1999). Moreover, the ApoE ε4 allele has a rate of 8% in India and China and this fact could account for the lower rate of AD found there compared to Western populations, because this allele seems to have the same association with AD in these countries as it does in Western countries (Ganguli, Chandra, Kamboh, Johnston, Dodge, et al., 2000; Liu, Hong, Wang, Fuh, Wang, et al., 1999). In Africa this relationship is less clear maybe because of different pressures including short life span. The ApoE ε3 is the most common in the Mayans of Central America (91%) and least common in the African pygmies (53%). The ApoE ε2 did not exist in the aboriginal Americans (Corbo & Scacchi, 1999; Fullerton, et al., 2000). Other authors, however, take a different prospective and claim that it is unlikely that the detrimental effects of ApoE ε4 in cardiovascular or neurological diseases might be significant enough to provide the necessary evolutionary pressure since these effects are post-reproductive. They suspect that there might be other reasons, such as an infectious disease selective pressure in selecting ApoE alleles such as a cataclysmic event in human history like the Great Plague which killed 30-50% of Europeans in the 14th century or smallpox (Mahley & Huang, 2009b). Finally, other speculative theories come from the inconsistent data obtained with child carriers of the ApoE ε4.

Turic et al., (2001) failed to observe statistically significant ApoE ε3 related differences in children in regard to general cognitive ability scores. Similarly, Deary et al., (2003) reported no statistically significant differences as a function of ApoE ε4 genotype on a test of general intelligence among 11-years-olds, although the magnitude of the group difference was larger than that observed in another study (Small, Rosnick, Fratiglioni, & Backman, 2004). However, Raber (2009) showed some influence of this allele on cognitive performance also in children, especially in those cognitive abilities strongly associated with brain regions more susceptible to AD type pathology. Taken together these results seem to indicate that ApoE ε4 is related to cognitive performance across the life span. Based on recent findings which have shown a different effect of ApoE isoforms across age, some authors suggest that a gene may have different functions at different stages of life (Reynolds, Prince, Feuk, Brookes, Gatz, et al., 2006; Nilsson, Adolfsson, Backman, Cruts, Nyberg, et al., 2006; Riley, Snowdon, Saunders, Roses, Mortimer, et al., 2000). In particular, the ε4 allele might have a positive effect on the organism in the early years of life with an exhaustive cost to the organism in later years. Williams (1957) called this phenomenon 'antagonist pleiotropy'. From an evolutionary point of view, therefore, a possible advantageous effect of the ApoE ε4 allele in childhood and adulthood could explain its existence and further persistence in humans. Lots of studies support this notion. ApoE ε4 has been associated with higher IQ scores (Yu, Lin, Chen, Hong, & Tsai, 2000), higher educational level (Hubacek, Pitha, Skodova, Adamkova, Lanska, et al., 2001) lower perinatal death (Becher, Keeling, McIntosh, Wyatt, & Bell, 2006), and reduced cardiovascular response to experimentally induced stress (Ravaja, Raikkonen, Lyytinen, Lehtimaki, & Keltikangas-Jarvinen, 1997). Hippocampal long-term potentiation (LTP) was enhanced at a young age in knock-in mice expressing human ApoE ε4 (Kitamura, Hamanaka, Watanabe, Wada, Yamazaki, et al., 2004). This enhancement was age related and disappeared in adult knock-in mice. On the other hand, there is evidence that ApoE ε4 alters intracellular calcium homeostasis, which might ultimately lead to neuronal damage (Qiu, Crutcher, Hyman, & Rebeck, 2003). Mondadori and colleagues (2007) have also speculated that an ApoE ε4 related neuronal calcium increase can be useful early in life but induce age-associated neuronal damage. Its association with cognitive impairment and abnormal brain activity later in life is likely mediated by AD-related preclinical neuropathology. In the past, life expectancy was lower, this effect of the ε4 allele was not observed but as life expectancy has increased, it is increasingly devastating to the human mind and body (Nilsson, et al., 2006). Obviously more research is needed to explore this gene-environment interaction that accounts for allele differences across different populations.

2.6.4 Genetics of ApoE in AD

2.6.4.1 *Effect of ApoE on Aβ aggregation and clearance*

Several mechanisms have been proposed to explain the effect of the ApoE ε4 allele on AD neuropathology. Evidence suggests that the major effect of the ApoE isoforms on the risk of developing AD is through its effect on Aβ aggregation and clearance, influencing the onset of Aβ deposition (Cole & Ard, 2000; Reiman, Chen, Liu, Bandy, Yu, et al., 2009).Other mechanisms, including the effects of the ApoE isoforms on synaptic function, neurotoxicity, tau hyperphosphorilation and neuroinflammation, may also contribute to the disease process (see Figure 2.8.).

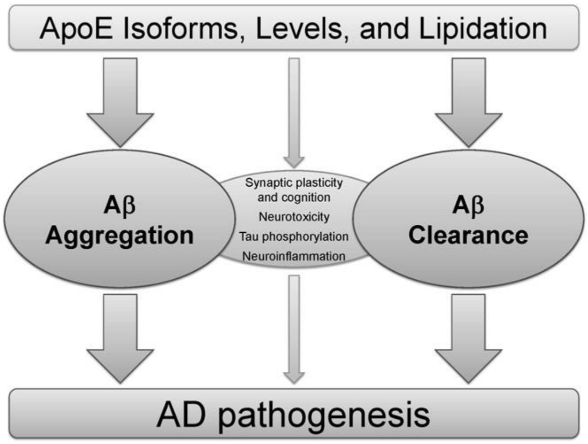


Figure 2.8. *ApoE isoform-specific effect on the risk of developing AD (Kim, Basak, & Holtzman, 2009)[Figure taken from (Kim, Basak, & Holtzman, 2009)].*

Many studies suggest a strong association between ApoE allele dosage and increased neuritic plaques in AD (Tiraboschi, Hansen, Masliah, Alford, Thal, et al., 2004). Furthermore, the level of Aβ42 in the CSF of healthy people at risk for AD has been founded to be ApoE ε4–dose (Sunderland, Mirza, Putnam, Linker, Bhupali, et al., 2004). Since people with brain amyloid deposition have low level of CSF Aβ42 (Fagan, Mintun, Mach, Lee, Dence, et al., 2006), this strongly suggests that amyloid deposition starts very early in people who are carriers of this mutation. Recently, Reiman (2009) showed that cognitively normal people had an ApoE ε4 dose-dependent increase in fibrillar Aβ burden in the brain as detected with an amyloid imaging agent. In addition to fibrillogenesis, there is evidence that ApoE alters both the transport and methabolism of Aβ in the brain. In cultured neuronal cells expressing the amyloid precursor protein, exogenous ApoE ε4 enhances Aβ production more than ApoE ε3 (Ye, Huang, Mullendorff, Dong, Giedt, et al., 2005). Interestingly, ApoE ε4 with Thr-61, which lacks domain interaction, acts like ApoE ε4 altering the structural composition of this allele, therefore converting its biological activity to one resembling ApoE ε3 (Mahley, et al., 2009a). ApoE ε4 seems to play a role even on the clearance of Aβ either by sequestering Aβ and modulating the cellular uptake of the ApoE-Aβ complex or by modulating Aβ removal from the brain into circulation by transport across the blood-brain barrier (Kim, et al., 2009). In addition to parenchymal deposits, most patients with AD also accumulate Aβ in cerebral blood vessels. This phenomenon is termed cerebral amyloid angiopathy (CAA). CAA is present in over 90% of patients with AD compared to 30% of the normal, aged population. One factor that might influence the relative accumulation of Aβ in cerebral blood vessels and in the parenchyma is the ApoE peptide, encoded by the ApoE gene. A number of studies suggest that differences in ApoE genotype influence both parenchymal and vascular deposition of Aβ. One study examined the relationship between the ApoE genotype and the relative extent of accumulation of Aβ as plaques within the cerebral parenchyma and in cortical blood vessels in the form of cerebral amyloid angiopathy (CAA), in autopsy brain tissue from 125 AD cases and from 53 elderly, neurologically normal controls of which 19 had CAA without other neuropathological features of AD (Chalmers, Wilcock, & Love, 2003). In the AD sample, the authors found that the severity of CAA was strongly associated with the number of ε4 alleles, but parenchymal Aβ load was independent of ApoE genotype. Cases with severe CAA had a lower parenchymal Aβ load than those with moderate CAA. These findings indicate that possession of the ApoE ε4 allele favours vascular over parenchymal accumulation of Aβ in AD. This may influence the pathogenesis of neurodegeneration in ε4-associated AD. A relationship between the ApoE genotype and the area of capillary basement membrane area was also found in patients with AD (Salloway, Gur, Berzin, Tavares, Zipser, et al., 2002). The quantitative evidence that microvascular changes were associated with ApoE genotype found in this study suggests a possible role for ApoE in the vascular changes present in AD. There was a statistically significant reduction in capillary basement membrane area (CBMA) in the ApoE ε4ε4 group compared to the ApoE ε3ε3 group, while CBMA decreased sequentially from ApoE ε3ε3 to ApoE ε3ε4 and to ApoE ε4ε4. The authors suggested that reduction in CBMA in ApoE ε4ε4 individuals is indicative of impaired blood-brain barrier (BBB) function, which might contribute to the pathogenesis of AD.

2.6.4.2 ***The effect of ApoE on Plasticity***

A number of studies suggest that ApoE is important for maintenance of neuronal plasticity and function. Studies in vitro showed isoform specific differences on neurite outgrowth (Nathan, Jiang, Wong, Shen, Brewer, et al., 2002; Teter, Xu, Gilbert, Roses, Galasko, et al., 1999). In vivo studies have shown findings consistent with those of in vitro studies. Compared with ApoE ε3 transgenic mice, those with ApoE ε4 had impaired compensatory sprouting and synaptogenesis after entorhinal cortex lesion, and were more severely impaired in learning and cognition (White, Nicoll, Roses, & Horsburgh, 2001; Raber, Wong, Buttini, Orth, Bellosta, et al., 1998; Raber, Wong, Yu, Buttini, Mahley, et al., 2000). In the presence of high level of Aβ and APP, several studies have also demonstrated that ApoE status affects synaptic plasticity and cognition in different ways (Raber, et al., 2000). Some other studies support evidence for a neuroprotective function of ApoE ε2 against Aβ-mediated toxicity. For example, dentritic spine loss observed in the hippocampus of young APP-trasgenic mice was ameliorated by ApoE ε2 overexpression (Lanz, Carter, & Merchant, 2003).

2.6.4.3 ***The role of ApoE in neurotoxicity***

ApoE synthesis can be induced by various stressor or stimuli (Xu, Walker, Bernardo, Brodbeck, Balestra, et al., 2008 Xu, et al., 2006) such as age, ischemia, trauma, Aβ deposition etc. Neuronal expression of ApoE is triggered to protect the cell and repair damage; however there are differences in how ApoE ε3 and ε4 cope with this function. ApoE ε4 synthesised by neurons undergoes proteolytic cleavage to a much greater extent than ApoE ε2 (Harris, Brecht, Xu, Tesseur, Kekonius, et al., 2003). The resulting fragments with C-terminal truncations escape the secretory pathway and enter cytosol; most are neurotoxic (Brecht, Harris, Chang, Tesseur, Yu, et al., 2004). Similarly, ApoE ε4 fragments are seen in the brains of transgenic mice expressing ApoE ε4 in neurons (NSE-ApoE) and in AD brains but not in transgenic mice expressing ApoE ε4 in astrocytes. These fragments accumulate with age in NSE ApoE ε4 mice, reaching a peak concentration at 6-8 months of age when these mice exhibit ApoE ε4 associated neuropathology and impaired learning and memory (Harris, et al., 2003).

2.6.4.4 ***Effect of ApoE isoform on Tau***

It is well known that hyperphosphorilation of the microtubule binding protein tau leads to NFTs which are one of the hallmarks of AD pathology. Although the physiological relevance of the direct interaction between ApoE and tau remains unclear, more recent data suggest the possibility that a fragment of ApoE ε4 (1-272 amino acids) escapes the secretory pathway, traslocates to the cytosolic compartment and interacts with cytoskeletal components including tau and neurofilament (Chang, ran Ma, Miranda, Balestra, Mahley, et al., 2005).

2.6.4.5 *ApoE and Nueroinflammation*

In vivo human ApoE knock-in mice studies suggest that ApoE ε4 may have a proinflammatory or less effective anti-inflammatory function and, therefore, may exacerbate or inefficiently prevent the detrimental neuroinflammation in AD, compared with the ApoE ε3 isoform, (Colton, Needham, Brown, Cook, Rasheed, et al., 2004; Guo, LaDu, & Van Eldik, 2004).

2.6.4.6 *The effect of ApoE on Metabolic Alterations in the brain*

Although most efforts focused on linking ApoE ε4 with the specific disease pathogenesis of AD within the brain, there is still the possibility that this gene indirectly affects AD onset and progression by modulating the function of the cerebrovascular system. In this regard studies showing the association between ApoE ε4 and cardiovascular disease (Song, Stampfer, & Liu, 2004) or arteriosclerosis support the possibility of a detrimental effect on brain function through decreased blood flow and altered metabolic properties. Positron emission tomography (PET) studies have shown that the AD brain exhibits decreased glucose metabolism in distinct regions (Alexander, Chen, Pietrini, Rapoport, & Reiman, 2002); this abnormal hypometabolism pattern has also been shown in healthy old and young carriers of the ApoE ε4 (Reiman, Chen, Alexander, Caselli, Bandy, et al., 2005; Reiman, Chen, Alexander, Caselli, Bandy, et al., 2004; Reiman, Caselli, Yun, Chen, Bandy, et al., 1996; Small, Ercoli, Silverman, Huang, Komo, et al., 2000).

In summary, prevailing data suggest that the main effect of ApoE isoform on the risk for AD is through Aβ metabolism, influencing the time of amyloid plaques onset in both parenchyma and vasculature. Since ApoE modulates not only the clearance but also the aggregation of Aβ and the neuropathological changes, it is difficult to elucidate the exact mechanisms resulting in Aβ phenotype. A critical issue is whether ApoE ε4 influences pathogenesis of AD by a gain of toxic function or a loss of protective function or both (Kim, et al., 2009). Due to this unresolved question, the two therapeutic strategies considered until now are to decrease the toxic effects of the ApoE ε4 in AD and targeting ApoE receptors such as LDLR and LPR.

2.6.5 ApoE related phenotype in AD

2.6.5.1 *Magnetic resonance imaging*

Although no certain diagnosis for AD can be made during life, structural imaging techniques can detect early volumetric changes predictive of dementia and functional imaging can detect changes in cerebral bloody flow and physiological and metabolic activity. In particular, brain imaging allows one to use the brain as a set of quantitative traits in genetic association studies (Hariri & Weinberger, 2003) .Although various findings suggest the AD process is pathologically and clinically heterogeneous (Binetti, Signorini, Squitti, Alberici, Benussi, et al., 2003;Holzer, Holzapfel, Zedlick, Bruckner, & Arendt, 1994) with a wide spectrum of cognitive, behavioural, biological and prognostic features that can vary between AD individuals, ApoE genotype status might contribute to the observed heterogeneity. As described above, this gene influences age of onset (Meyer, et al., 1998) and might act by modulating the pathological process underlying the disease, increasing cortical and cerebrovascular amyloid deposits (Gomez-Isla, Price, McKeel, Morris, Growdon, et al., 1996; Schmechel, Saunders, Strittmatter, Crain, Hulette, et al., 1993), the number of neurofibrillary tangles (Beffert & Poirier, 1996; Schmechel, Saunders, Strittmatter, Crain, Hulette, et al., 1993) and increasing cholinergic deficits (Beffert & Poirier, 1996). Moreover ApoE related clinical heterogeneity after AD onset is supported by observations reporting that the presence of the ε4 allele is associated with an increased risk of psychosis (Scarmeas, Brandt, Albert, Devanand, Marder, et al., 2002), a lower risk for developing extrapiramidal signs, lower rate of cognitive decline and lower mortality (Stern, Tang, Albert, Brandt, Jacobs, et al., 1997).

There is a controversy regarding the ApoE effects on the atrophy of brain tissue. Post mortem studies have reported greater accumulation of AD pathological hallmarks in the neocortex of patients carrying the ε4 allele than those without the ε4 allele (Tiraboschi, et al., 2004). In vivo data, however, seem to agree only partially with this result. Recently, Pievani et al., (2009) have shown increased susceptibility of the temporal cortex and together with lower vulnerability in the frontal-parietal neocortical regions in AD ε4 carriers compared to non-carriers. A recent meta-analysis computed a mean annual hippocampal atrophy rate of 4.7% for AD participants and 1.4% for controls (Barnes, Bartlett, van de Pol, Loy, Scahill, et al., 2009). Lehtovirta (1995; 1996) found that normal hippocampal “right bigger than left” asymmetry was diminished in non-demented elderly subjects [see also (Soininen, Partanen, Pitkanen, Hallikainen, Hanninen, et al., 1995)] and the hippocampal and amygdala damage was greater in AD patients carrying the ε4 allele despite equal global cognitive severity; they also found larger frontal volume. One year later they replicated the study with a bigger sample finding again significantly smaller MLT structures in carriers than non-carriers (Soininen & Riekkinen, 1996). Juottonen (1998) extended the findings of a greater atrophy of the MLT to the entorhinal cortex in AD carriers compared to non-carriers. These results were replicated by Geroldi et al., (Geroldi, Pihlajamaki, Laakso, DeCarli, Beltramello, et al., 1999) who showed greater hippocampus, entorhinal cortex (ERC) and temporal lobe atrophy for AD ε4 carriers and larger frontal volume in the same subgroup of patients. More recently, other studies have confirmed these results and found smaller hippocampus, amygdala and entorhinal cortex in AD ε4 carriers (Agosta et al., 2009; Filippini, Rao, Wetten, Gibson, Borrie, et al., 2009; Boccardi, Sabattoli, Testa, Beltramello, Soininen, et al., 2004; Hashimoto, Yasuda, Tanimukai, Matsui, Hirono, et al., 2001). Agosta et al.,(2009) found strongest ε4 effect in neocortical regions (parietal region bilaterally, precuneus and dorsolateral prefrontal cortex), maybe due to the young AD sample they studied in their experiment and more hippocampal atrophy in ε4 AD than non-carriers despite equal cognitive performance. Again, Muller et al., (2008) showed a regionally selective effect of the ε4 on cornu ammonis sector 3 and dental gyrus (CA3&DG) of the hippocampus fields and smaller enthorinal cortex. However, another group of authors showed no significant smaller hippocampal volumes in either controls or AD patients carrying the ε4 allele, even though there was a trend for smaller volume in the ε4 carriers in both of their groups (Jack, Petersen, Xu, O'Brien, Waring, et al., 1998b). Moreover, Yasuda et al., (1998) in a study with 178 AD patients found a larger brain in an ε4–dose dependent manner. Finally, the majority of the longitudinal studies have also shown inconsistent data (Jack, et al., 1998b; Tanaka, Kawamata, Shimohama, Akaki, Akiguchi, et al., 1998; Yamaguchi, Nakagawa, Arai, Sasaki, Higuchi, et al., 1996).

2.6.5.2 ***White matter change***

Not many studies have focused on white matter changes detected with MRI in relation to ApoE ε4 in prediction of mild cognitive impairment and in AD. However, the results have been inconsistent, with no significant association between ApoE and white matter changes in some instances and deep white mater lesion in others (Changsheng Wang, Stebbins, Medina, Shah, Bammer, et al., 2010; Smith, Egorova, Blacker, Killiany, Muzikansky, et al., 2008; Doody, Azher, Haykal, Dunn, Liao, et al., 2000; Bronge, Fernaeus, Blomberg, Ingelson, Lannfelt, et al., 1999; Amar, MacGowan, Wilcock, Lewis, & Scott, 1998).

2.6.5.3 ***Cerebral perfusion and metabolism***

There are other studies that have focused their attention on the relationship between regional cerebral blood flow (rCBF) or glucose metabolism (rCMRgl) and ApoE genotype. PET studies showed abnormal precuneus, posterior cingulate, parietotemporal and frontal low cerebral metabolism rate for glcose (rCMRgl) in AD (Alexander, et al., 2002; Minoshima, Frey, Koeppe, Foster, & Kuhl, 1995). However, Lehtovirta et al., (1996) using SPECT to study rCBF in AD patients, found a tendency toward higher levels of perfusion in the frontal lobe as a function of the numbers of alleles. A similar finding was reported by Higushi et al., 1997). Other studies found no rCMRgl differences between AD carriers and non-carriers (Corder, Jelic, Basun, Lannfelt, Valind, et al., 1997; Hirono, Mori, Yasuda, Ishii, Ikejiri, et al., 1998).

Although all the studies are not in complete agreement, evidence suggests that the ApoE genotype in AD exerts a differential effect on regional brain volumes. Different theoretical models have been formulated in attempt to explain this heterogeneity. As for the regional selective effect of ApoE ε4 in the hippocampus, an autopsy study showed increased neurogenesis and new neuron formation in hippocampal fields in reaction to the pathological processes in AD (Jin, Peel, Mao, Xie, Cottrell, et al., 2004). Since cholesterol metabolism is necessary for neurogenesis, it seems plausible that this compensatory process could be differently affected by ApoE ε4 status. Levi et al., (2007) showed increased apoptosis and reduced neuronal density in response to environmental enrichment in ApoE ε4 transgenic mice, but neurogenesis in ApoE ε3 transgenic mice. Another issue has arisen from these results and concerns the functional consequences of these regional differences in brain atrophy. In particular, non-significant, although evident, differences in cognitive performance in carriers and non-carriers despite more pronounced hippocampal atrophy has been found (Mosconi, Nacmias, Sorbi, De Cristofaro, Fayazz, et al., 2004). A possible explanation could be due to the relatively preserved frontal lobe shown in carriers in the early stage of the disease. This compensatory speculation is consistent with AD neuropsychological studies which show poorer temporal function and better frontal cognitive functions in patient (Venneri, Forbes-Mckay, & Shanks, 2005; Lehtovirta, et al., 1996) (see Chapter 3).

2.6.6 ApoE related phenotype in MCI

To date, a large body of evidence has already showed that cognitive deficits and neuropathological hallmarks of the disease can be detected very early in the course of AD or even before the stage of clinical onset. In this regard, those with amnestic Mild Cognitive Impairment (aMCI), who seems to represent a preclinical stage of AD along the continuum between health and established disease, have become the object of investigation for most researchers. Comparison of aMCI patients to healthy controls and AD have revealed different patterns of atrophy, depending on the type and state of cognitive impairment (Chetelat, Desgranges, de la Sayette, Viader, Eustache, et al., 2003; Chetelat, Landeau, Eustache, Mezenge, Viader, et al., 2005; Whitwell, Petersen, Negash, Weigand, Kantarci, et al., 2007) . Among the prodromical symptoms, marked neuronal loss in the medial and anterior temporal regions as the presence of neurofibrillary tangles and senile plaques can be observed very soon in the course of the disease (Gottesman & Gould, 2003). Similar to the findings of volumetric MRI studies which have shown hippocampal atrophy before the onset of the dementia (Jack, Jr., Petersen, Xu, O'Brien, Smith, et al., 1999; Visser, Scheltens, Verhey, Schmand, Launer, et al., 1999a; Fox, Warrington, Stevens, & Rossor, 1996a) and progressive deterioration along the continuum toward the disease (Fox, Warrington, Freeborough, Hartikainen, Kennedy, et al., 1996b), Risacher et al., (2009) recently used VBM and automated parcellation and found local atrophy (bilateral MLT) in AD and MCI in a large cohort compared to controls. Similar findings were obtained in other recent studies which used different techniques (TBM, RAVENS) (Misra, Fan, & Davatzikos, 2009; Hua, Leow, Parikshak, Lee, Chiang, et al., 2008a) and in other studies carried out with small samples (Jack, Jr., Bernstein, Fox, Thompson, Alexander, et al., 2008; Hamalainen, Grau-Olivares, Tervo, Niskanen, Pennanen, et al., 2008; Pennanen, Testa, Laakso, Hallikainen, Helkala, et al., 2005). Interestingly, significantly different atrophy patterns between MCI converter and MCI stable patients has been found, with the former showing more similar structural brain changes (hippocampal, entorhinal cortex, amygdalae and other MTL volume estimates) to AD than the latter. These findings again support previous results obtained with smaller samples (Devanand, Liu, Tabert, Pradhaban, Cuasay, et al., 2008; Fleisher, Sun, Taylor, Ward, Gamst, et al., 2008; Karas, Sluimer, Goekoop, van der Flier, Rombouts, et al., 2008; Tapiola, Pennanen, Tapiola, Tervo, Kivipelto, et al., 2008; Whitwell, Shiung, Przybelski, Weigand, Knopman, et al., 2008; Bozzali, Filippi, Magnani, Cercignani, Franceschi, et al., 2006a; Bell-McGinty, Lopez, Meltzer, Scanlon, Whyte, et al., 2005; Chetelat, et al., 2005) . PET studies have showed hypometabolism of limbic areas in aMCI and AD with extended hypometabolism outside the limbic network for the AD patients (amygdala and lateral cortical region) (De Santi, de Leon, Rusinek, Convit, Tarshish, et al., 2001; Minoshima, Giordani, Berent, Frey, Foster, et al., 1997).

This finding provides support for the notion that atrophy in limbic structures is the first significant event in the evolution of AD (Anchisi, Borroni, Franceschi, Kerrouche, Kalbe, et al., 2005; Nestor, Fryer, Smielewski, & Hodges, 2003; De Santi, et al., 2001). Finally, longitudinal studies have demonstrated that baseline CMRglc (cerebral metabolic rate for glucose) reduction is more pronounced in MCI who progress to AD than in those who remain stable (Anchisi, et al., 2005; Drzezga, Grimmer, Riemenschneider, Lautenschlager, Siebner, et al., 2005; Chetelat, et al., 2003).

In summary the most common finding is that of decreased grey matter volume in the medial temporal regions, posterior and neocortical part of the temporal lobes, posterior cingulate, precuneus and frontal areas (Stoub, deToledo-Morrell, Stebbins, Leurgans, Bennett, et al., 2006; Whitwell, et al., 2008; Chetelat, et al., 2005) with smaller grey matter density in medial temporal regions in MCI ε4 carriers compared to non-carriers (Thomann, Roth, Dos Santos, Toro, Essig, et al., 2008). Van de Polet al., (2007) carried out an MRI study with 323 MCI individuals who were followed up for 2 years in a clinical trial and found that MCI carriers showed a more severe rate of hippocampal volume loss (-3.6% per year) than non-carriers. These findings are consistent with neuroimaging data which show areas of atrophy in MCI ε4 carriers compared to non-carriers in medial temporal regions, with greater atrophy in the parahippocampal gyrus, amygdala and thalamus (Pennanen, Testa, Boccardi, Laakso, Hallikainen, et al., 2006), and abnormal activation in the thalamus and medial temporal structures during verbal paired associated learning (Bassett, Yousem, Cristinzio, Kusevic, Yassa, et al., 2006).

2.6.7 ApoE related phenotype in healthy subjects

Imaging techniques are an adjunctive screening measure for undetected pathology and represent an important expanding field in biological neuropsychiatry (Knopman, DeKosky, Cummings, Chui, Corey-Bloom, et al., 2001). Structural imaging techniques can detect early volumetric changes predictive of dementia, and functional imaging can detect preclinical changes in cerebral blood flow, metabolic activity, and neurotransmitter and receptor function. There has been a variety of brain changes associated with the ApoE allele 4 in AD, including structural and functional brain changes. Furthermore, studies suggest that the pathological burden of AD might begin decades prior to the diagnosis of AD and may be influenced by one’s ApoE genotype [e.g. (Bondi, Houston, Eyler, & Brown, 2005; Reiman, et al., 2005; Scarmeas, Habeck, Stern, & Anderson, 2003)].

2.6.7.1 ***Functional brain imaging.***

Fluorodeoxyglucose positron-emission tomography (FDG-PET) studies examining the effects of the ApoE ε4 allele on CMRglc in non-demented individuals (from young age to middle age) have reported that compared to non-carriers, ε4 carriers have mild but definite CMRglc reductions in the same regions usually affected by neuropathology in patients with established AD.

ApoE-related brain function during rest

In a study of non-demented middle-age subjects with memory complaints who had at least two relatives with AD, 12 participants carring ε4 and 19 non-carriers participants were scanned with fluorodeoxyglucose positron-emission tomography (FDG-PET) (G. W. Small, Mazziotta, Collins, Baxter, Phelps, et al., 1995). Carriers did not differ from non-carriers in mean age or in neuropsychologic performance. Parietal metabolism was significantly lower, and left-right parietal asymmetry was significantly higher in carriers, as compared with non-carriers. The same investigators reported similar results from a FDG-PET study that included 27 ε4 carriers and 27 ε4 non-carriers who were elderly, dementia-free and who had memory complaints and/or family history of dementia (Small, et al., 2000). The analyses (ROI and SPM) showed that ε4 carriers had significantly lower metabolism in bilateral inferior parietal regions, poster cingulate and left lateral temporal areas, compared with ε4 non-carriers. After 2 years a significant metabolic decline was noted for ε4 carriers in the same areas but not for the ε4 non-carriers, who showed metabolic decline primarily in the frontal cortex, consistent with normal aging. As part of a study of cognitively normal late middle-age subjects with family history of probable AD, FDG-PET images were acquired in 11 ε4 homozygotes and 22 control subjects without the ε4 allele who were matched for gender, age, education and level of cognitive performance (Reiman, et al., 1996). The ε4 homozygotes had significantly reduced rates of glucose metabolism in the same posterior cingulate, parietal temporal and prefrontal regions as previously found in studies of patients with probable AD. The same investigators observed similar results with a larger dataset of 160 cognitively normal late middle-age subjects who had a first-degree relative with AD (Reiman, et al., 2005). In another report from the same group, longitudinal PET cerebral glucose metabolic data after 2 years were available for 10 cognitively normal ε4 heterozygotes and 15 non-carriers, 50 to 63 years of age, with a reported family history of AD (Reiman, Caselli, Chen, Alexander, Bandy, et al., 2001). The ε4 heterozygotes had significant cerebral metabolism declines in the vicinity of temporal, posterior cingulate, and prefrontal cortex, basal forebrain, parahippocampal gyrus, and thalamus, and the declines were significantly greater than those noted for ε4 non-carriers.

The association between ApoE genotype and functional brain imaging changes in young subjects has been investigated by a few studies. In one, FDG-PET scans were obtained from normal volunteers 20 to 39 years of age (12 ε3ε4 and 15ε3ε3/ε2ε3) (Reiman, et al., 2004). Similar to previously studied patients with probable AD and late middle-aged ε4 carriers, the young ε4 carriers had abnormally low rates of glucose metabolism bilaterally in the posterior cingulated, parietal, temporal and prefrontal cortex. Another study used H_2(15)O PET to measure cerebral blood flow of 18 younger college-age subjects (three were carriers for the ε4 allele while the other fifteen did not have the allele) (Scarmeas, et al., 2003). The carriers were of similar age (26 versus 23 years, p = .07), education (17.3 versus 16.5 years, p = .56) and sex (p=.13) to non-carriers. Neuropsychological performance didn't differ between the groups measured with the MMSE. Compared with individuals without the ε4 allele, ε4 carriers exhibited significantly lower resting cerebral blood flow rCBF in the left and right inferior temporal gyri while resting rCBF was higher in the left insula, right supramarginal gyrus, and the inferior occipital gyrus. It is interesting how the observed significant association in these studies indicates that despite the small number of individuals in the ε4 group (which is consistent with the proportions of the ApoE polymorphisms in the population), the effects were strong enough to be demonstrable. Finally, a recent longitudinal study, comparing changes in rCBF over a 8 year period between 29 non-demented ApoE ε4 carriers and 65 non-carriers older than 55 years showed greater decline in ε4 carriers in the same areas affected by early AD neuropathological changes (Thambisetty, Beason-Held, An, Kraut, & Resnick, 2010).

Decline in brain function explains these changes resulting from multiple effects due to the ApoE ε4 allele. It could be that impaired brain repair mechanisms in carriers (Arendt, et al., 1997) make specific regions more vulnerable to the effect of environmental risk factors. Thambisetty et al., (2010) observed higher baseline rCBF values in ApoE ε4 carriers in those regions exhibiting longitudinal rCBg decline. It could be that compensatory mechanisms in carriers are responsible for that and explain the equal cognitive performance in both groups despite functional brain changes. Findings from other study is consistent with this hypothesis (Scarmeas, et al., 2003). Alternatively these changes could also reflect early neuropathological changes (β-amyloid deposition and sNFT) in carriers of the ε4 allele; areas particularly vulnerable to the pathological changes very early in the course of the AD.

ApoE-related alteration in brain function

fMRI research investigating the role of the ApoE in different cognitive domains in cognitively intact adults suggests that the presence of an ε4 allele is associated with up-regulation of brain activity in areas associated with episodic, semantic and working memory. Wishard et al., (2006) showed greater brain activation in medial and dorsolateral prefrontal cortex and parietal regions bilaterally during a working memory task in adults heterozygous for the ApoE ε4 than has seen in ε3ε3 individuals. Fleisher et al., (2005a) showed up-regulation in neurons in areas supporting memory systems in people at risk for AD (10 ε4 carriers) compared to non-carriers especially in regions associated with early development of AD pathology (medial temporal lobe). Bookheimer et al., (2000) found that during a word recall task, non-demented allele ε4 carriers had greater activation than did non-carriers in the left prefrontal region and bilateral orbitofrontal, superior temporal, and inferior and superior parietal regions. These abnormal patterns of activation may represent a compensatory functional response. That is, the use of additional brain resources to perform the task.

The increased activations in the allele ε4 carriers (detected for various levels of task difficulty) were specific to memory, and not related to general task difficulty (Bookheimer, et al., 2000). In one fMRI study, Smith and colleagues (Smith, Andersen, Kryscio, Schmitt, Kindy, et al., 1999) examined cortical activation in two groups of cognitively normal middle-aged women who differed only in terms of their

AD risk (i.e., family history of AD and ApoE status). The groups performed a visual naming and a letter fluency task during scanning. High risk women showed lower levels of regional activation in bilateral mid and posterior inferotemporal regions during naming and fluency tasks despite identical behavioural performance. In a follow-up study of these women participants by the same group of investigators, the high-risk group showed significantly decreased activation in the left posterior fusiform and bilateral anterolateral occipital areas (Smith, et al., 2002; Smith, Kryscio, Schmitt, Lovell, Blonder, et al., 2005).

The main findings of the fluency fMRI task appear to be that normal individuals at high risk for AD demonstrate a significantly increased parietal activation when compared with a matched group of low-risk individuals performing the same letter fluency task. This increased activation was present many years before the time of which clinical symptoms of AD typically appear. The high-risk individuals were indistinguishable from their low-risk counterparts in several cognitive measures, including verbal fluency. The location of this region was also adjacent to that observed by others using a recall task. The combined evidence from both studies suggests, therefore, a disruption of functional circuits involving the left parietal lobe in asymptomatic individuals at increased risk for AD. Different tasks may reveal this disruption by probing specific parietal sub-circuits according to their different processing demands. Increased activation in the high-AD-risk group could be due to the relatively increased cognitive work needed in individuals possessing brain networks compromised by in-situ AD pathology.

Another study, examined the effect of ApoE genotype on brain activation patterns in the MLT during an episodic encoding task in cognitively normal individuals with a family history of AD who were on average 15-20 years younger than the age at which AD symptom typically develop (Trivedi, Schmitz, Ries, Torgerson, Sager, et al., 2006). The authors found that ε3/ε4 heterozygotes displayed reduced fMRI activation compared to allele ε3/ε3 homozygotes in the right hippocampus and entorhinal cortex during encoding of novel relative to the encoding of familiar pictures. There were also no significant differences between the groups in age, education or memory function, and neuropsychological performance was within the normal range for both groups. This suggest that reduced MTL activation for novel items in allele ε3/ε4 heterozygotes was not caused by impaired cognitive function, and that the observed neurobiological changes in MTL function precede the onset of measurable decline in cognitive function. These authors also found no evidence for differences in regional grey matter volume as measured by voxel based morphometry, suggesting that the observed activation differences were not caused by reduced MTL GM volume on their cohort of middle-aged subjects, further supporting the notion that reduced fMRI activation in ε3/ε4 heterozygotes preceded overt changes in hippocampal volume. Lind et al., (2006) found that 30 cognitively intact carriers of the ApoE ε4 allele (10 ε4/ε4, 20 ε3/ε4) had reduce functional brain activity in the left parietal cortex and bilaterally in the anterior cingulated region during semantic categorization task, compared to 30 non-carriers.

Another study used $H_2(15)O$ PET for imaging 26 non-ε4 carriers and six elderly ε4 carriers while they did a serial recognition non-verbal memory task with titrated task difficulty so that recognition accuracy was similar for all subjects (Scarmeas, Habeck, Anderson, Hilton, Devanand, et al., 2004a). Compared with non-ε4 carriers, ε4 carriers showed significantly decreased activation in the left precuneus, left superior temporal, right superior frontal, left postcentral and posterior cingulate gyri. Because recognition accuracy was titrated, the differences in activation were considered not to reflect task difficulty, but to indicate memory-related altered cognitive processing in the different ApoE genotypes.

A recent fMRI study found that non-demented older adult carrying the ApoE ε4 allele with normal learning and memory capabilities showed greater magnitude and extent of BOLD brain response during picture learning relative to their ε3 counterparts in multiple brain regions (e.g. bilateral fusiform and medial frontal gyri, left inferior and middle frontal, right superior parietal, and right hippocampal and parahippocampal cortices) (Bondi, et al., 2005). In addition, the ApoE ε4 group showed lower brain response in the left hippocampus, relative to the ApoE ε3 group.

Both groups were performing equally well across many learning and memory measures. Both groups demonstrated comparable volumes of grey matter and white matter and CSF, all of which were also quite consistent with grey, white and CSF segmentation volumes of older adults reported by other (Courchesne, Chisum, Townsend, Cowles, Covington, et al., 2000). The absence of volumetric differences between the at-risk sample and the control, in the face of functional differences, is in keeping with previous reports distinguishing functional and structural differences. For example, Remain et al., (1996; 1998b), using both resting PET and MRI, contrasted 11 individuals with APOE ε4/ε4 with 22 individuals with APOE ε3/ε3 and found that while the ε4 allele was associated with decreased metabolism, particulary in the cingulum, there were no volumetric differences, demonstrating that functional differences occur in the absence of volumetric loss. It appears, therefore, that volumetric loss is discernable only in the presence of cognitive decline, which is absent in an at-risk sample (Wolf, Grunwald, Kruggel, Riedel-Heller, Angerhofer, et al., 2001). Recent imaging work has also suggested that surface deformity of the hippocampus, rather than volume of the hippocampus, may be predictive of subsequent cognitive decline and onset of Alzheimer's disease (Csernansky, Wang, Swank, Miller, Gado, et al., 2005).

Moreover, fMRI studies also confirm evidence of compensatory hypothesis where ApoE ε4 persons appear to require additional cognitive effort to achieve the same level of performance. Indeed, several functional neuroimaging studies have shown that BOLD brain responses associated with the performance of memory tasks is more diffuse in patients with early AD than in normal older individuals, suggesting recruitment of areas outside of the usual structures that mediate memory (Grady, McIntosh, Beig, Keightley, Burian, et al., 2003; Becker, Mintun, Aleva, Wiseman, Nichols, et al., 1996; Sperling, Bates, Chua, Cocchiarella, Rentz, et al., 2003). It may be that, after an initial decline in memory proficiency following damage to MTL structures, patients in the preclinical stage of AD are able to effectively recruit compensatory brain resources (e.g. frontal and temporal cortical regions important for executive function and semantic memory) to halt or slow further memory decline for a period of time. A similar compensatory response in certain brain-derived neurotrophic factors (Egan, Kojima, Callicott, Goldberg, Kolachana, et al., 2003; Durany, Michel, Kurt, Cruz-Sanchez, Cervos-Navarro, et al., 2000) or cholinergic activity (DeKosky, Ikonomovic, Styren, Beckett, Wisniewski, et al., 2002) may also attenuate memory changes for a time. Given that adequate cholinergic activity and neurotrophic mechanisms are partly responsible for the maintenance of neuronal function and structural integrity, these findings suggest that, under conditions of progressive neurodegeneration, the MTL stimulates the overexpression of certain cholinergic and neurotrophic factors as possible mechanism of compensation. As the disease progresses, however, each of these additional resources becomes compromised and patients exhibit a period of rapid decline in episodic memory abilities (Lange, Bondi, Salmon, Galasko, Delis, et al., 2002). Decline in brain activity (Sperling, et al., 2003) and cholinergic activity (DeKosky, et al., 2002) in brain regions critical for episodic memory function would then be expected in clinically evident AD.

In summary, the cognitive and neuroimaging changes of incipient AD appear to remain relatively stable until a few years before clinical diagnosis, when there is a more notable decline. The mild course of decline in the early preclinical period may reflect the initial involvement of compensatory brain resources to overcome the accrual of plaques, tangles, and neuron and synapse losses. A growing body of fMRI evidence from at-risk people supports this notion (Han, Houston, Jak, Eyler, Nagel, et al., 2007; Bondi, et al., 2005; Dickerson, Salat, Greve, Chua, Rand-Giovannetti, et al., 2005; S. Y. Bookheimer, et al., 2000; Johnson, Vogt, Kim, Cotman, & Head, 2004), and a similar compensatory response in brain-derived neurotrophic factors (Egan, et al., 2003; Durany, et al., 2000) or cholinergic activity (DeKosky, et al., 2002) may also occur.

Given these converging lines of evidence for brain compensation, Twamley et al., (2006), propose a non-linear model of episodic memory decline and neuroimaging changes to characterise this preclinical period of accruing AD pathology. According to these authors cognitive and neuroimaging changes of AD remain relatively stable because of compensatory mechanisms until a few years before clinical diagnosis where it’s possible to see a decline due to the failure of these resources to compensate for the extensive neurodegenerative damage. Consistent with this model, Martins et al., (Martins, Oulhaj, de Jager, & Williams, 2005) have demonstrated that possession of ApoE ε4 allele is associated with earlier and faster cognitive decline in patients with AD, whereas the ε2 allele is related to slower decline, and that a non-linear model best predicts these differential rates of decline.

In another study, using H_2(15)O PET, 20 healthy young adults (age 19-28) were scanned while they performed a non-verbal memory task (Scarmeas, Habeck, Hilton, Anderson, Flynn, et al., 2005a). Compared to subjects without the ε4 allele, ε4 carriers showed significantly lower activation in right superior temporal and left fusiform gyri and significantly higher activation in left middle temporal and right transverse temporal gyri. Mondadori et al., (2007) carried out an fMRI study with 37 young participants (mean age 22.8) and showed lower levels of brain activity during learning and retrieval in the right middle and superior frontal gyri and right precuneus during face retrieval and right hippocampus and left fusiform gyrus during associative retrieval in ε4 carriers compared to non-carriers (ε3/ε3, ε3/ε2), despite equal behavioural performance. These results suggest that ApoE-dependent modulation of cerebral flow during cognitive activation may be present even at a very young age (see section 2.6.3.).

Recently some studies have focused their attention on the “resting state networks” (RSNs) and how it can be affected by the ApoE ε4 allele. RSNs reflect spontaneous low fluctuations (less than 0.1 Hz) in resting brain function. It is also called the “default mode network” (DMN) and includes the prefrontal, anterior and posterior cingulate, lateral parietal, inferior/middle temporal gyri; cerebellar areas; thalamic nuclei and mesial temporal regions (MTL) (Boly, Phillips, Tshibanda, Vanhaudenhuyse, Schabus, et al., 2008). The DMN is affected by neurodegenerative processes (Bukner 2005), both AD patients (Greicius, Srivastava, Reiss, & Menon, 2004) and MCI individuals (Sorg, Riedl, Muhlau, Calhoun, Eichele, et al., 2007) are reported to show reduced coactivation of hippocampal and posterior cingulate regions. Filippini et al., (2009a) carried out a study with 18 young ApoE ε4 carriers and 18 non-carriers (age 20-35) showing increased hippocampal coactivation within the DMN during rest and greater hippocampal activation during memory encoding task in the absence of any difference in brain volume, resting brain perfusion or memory performance. Fleisher et al., (2009), using a similar design, found abnormalities in resting-state networks and lower performance in semantic fluency task in 17 carriers compared to 12 non-carriers. As described before, greater activation in ApoE ε4 carriers has been interpreted as a possible greater cognitive effort by ε4 carriers to obtain the same level of performance as non-carriers. However it is unlikely that this interpretation may be applicable to explore the patterns observed in young ε4 carriers who do not have any manifest cognitive decline. Some authors posit the presence of neuronal mechanisms such as reduced synaptic plasticity, neuronal growth or altered long-term potentiation (LTP) as the factors that may be responsible for those results in people carrying the ApoE ε4 allele (Bellosta, Nathan, Orth, Dong, Mahley, et al., 1995; Buttini, Orth, Bellosta, Akeefe, Pitas, et al., 1999; Trommer, Shah, Yun, Gamkrelidze, Pasternak, et al., 2005).

2.6.7.2 ***Structural brain imaging***

The relationship between ApoE genotype and morphometric indices appears to be a complex one, with regionally specific alterations for a given allele combination and age-dependent effects (Bondi, Houston, Salmon, Corey-Bloom, Katzman, et al., 2003; Caselli, Osborne, Reiman, Hentz, Barbieri, et al., 2001). A number of studies have reported volumetric differences in the hippocampus (which did not reach significance however) between cognitively intact ApoE ε4 carriers and non-carriers

(Jack, Petersen, Xu, O'Brien, Waring, et al., 1998; Reiman, Uecker, Caselli, Lewis, Bandy, et al., 1998a). Plassman et al., (1997) found a significantly different hippocampal volume in non-demented adult ε4 carriers compared to controls, despite no difference in neuropsychological measures.

Clear ApoE-related changes have been found in difference in brain regions measured by computed tomography (CT) imaging scan analysis in a Spanish population with dementia. In general, ApoE ε4 carriers show an anticipated age-dependent brain atrophy reflected by increase in both the interventricular distance and the interhippocampal distance which is more significant in AD than in an aged population (Etcheverria, Amado, Pichel, & Cacabelos, 2003).

Ridha at al., (2006) showed that atrophy of the whole brain and the hippocampus, as seen with serial MRI scans, predates the clinical diagnosis of Alzheimer's disease. These authors showed that by the time the disease was diagnosed clinically the estimated mean hippocampal volume in patients was 18.1% smaller and mean whole-brain volumes were 5.4% smaller than in controls. The results suggest an association between clinical stages of AD and volumetric measurements. As mutation carriers moved from presymtomatic to MCI and AD stages, mean total hippocampal and whole-brain volumes decreased. Additionally, rates of whole brain and hippocampal atrophy gradually increased with a mean rate of around 0.5% per year and 3% per year, respectively in the transition through the MCI stage. This finding lends support to the view that MCI represents an early stage of AD.

Another study show reduced right hippocampal volume in ApoE ε4 carriers that ranged in age between 49 and 79 years (Lind, et al., 2006). These authors found that the difference in hippocampal volume between carriers and non-carriers was most pronounced before the age of 65. Thereafter, the difference was attenuated, possibly as a function of age-related hippocampal atrophy.

In an MRI study of 113 cognitively normal, late middle-aged adults, the presence of the ε4 allele had a dose-related reduction (i.e. more prominent reduction for ε4 homozygotes, as compared with ε4 heterozygotes, and for ε4 heterozygotes as compared with non-ε4 carriers) of grey matter in regions known to be affected early in AD, including the posterior cingulate, bilateral para-hippocampal/lingual gyri, and left parietal and anterior cingulate/medial frontal areas (Alexander, Chen, & Reiman, 2003). In a MRI study of 32 healthy elderly subjects (16 with age-associated memory decline, and 16 with normal memory), ε4 homozygotes had significantly smaller right (as compared to left) hippocampus (Soininen, et al., 1995). This pattern of hippocampus asymmetry was opposite to that observed in non-ε4 carriers (i.e. larger right hippocampus).

These results were interpreted as concordant with studies of established AD, indicating a more severe volume loss in the right hippocampus for ε4 homozygotes (Lehtovirta, et al., 1995). Another MRI study included 193 subjects (68 ε3ε3, 102 ε3ε4, 23 ε4ε4) with MCI and compared them with non-ε4 carriers. Women with one or two ε4 alleles had significantly reduced hippocampal volume, whereas in men a significant reduction in hippocampal volume was found only for those carrying two ε4 alleles (Fleisher, et al., 2005a). When controlled for memory performance on a delayed word recall task, the ApoE effect on hippocampal volume was attenuated in men, but remained significant in women. These authors concluded that the ApoE genotype status seems to have a greater deleterious effect on gross hippocampal pathology in women than in men.

Lemaitre et al., (2005) showed that healthy ε4 homozygotes had greater hippocampal atrophy than heterozygotes and non-carriers with associated risk of cognitive impairment (increased by a factor of 6 in homozygotes but didn't differ between heretozygotes and non-carriers). Whishard et al., (2006a) found reduction in the frontotemporal regions and in the right medial temporal lobe (MTL) in healthy ε4 carriers compared with non-carriers. In a study of 70 healthy paediatric subjects who were scanned with brain MRI at the age of 10 years, there was no relation between ApoE status and hippocampal volume (Scarmeas & Stern, 2006). For 50 of these subjects who were scanned aged 2 years later, there was no association between ApoE status and longitudinal changes in hippocampal volume. Chen et al., (2007) found a significantly higher rate of whole brain atrophy in cognitively normal carriers than in non-carriers. Significant differences in cortical thickness of hippocampal subregions (in entorhinal cortex and subiculum but not volumetric difference in the hippocampus or perirhinal cortex) have been reported in 14 cognitively normal ε4 carriers (Burggren, Zeineh, Ekstrom, Braskie, Thompson, et al., 2008). Similarly, selective regional effects of the ApoE ε4 genotype on the subfield CA3 and the dentate gyrus of the hippocampus in normal aging and AD were reported by another study (Mueller, et al., 2008). Interestingly, Shaw et al., (2007), when comparing the MRI scans of 239 healthy children and teenagers, confirmed thinner entorhinal cortex in carriers than in non-carriers, with ε2 carriers showing the thickest enthorhinal cortex. Recently, Crivello et al., (2010) in an MRI study with 1186 healthy elderly participants found that the rate of hippocampal atrophy is not relevant for distinguishing heterozygote subjects from non-carriers and shouldn't, therefore, be used as a preferred phenotype. Finally, Honea et al., (2010) found a cognitive performance reduction associated with atrophy in hippocampus and amygdala and decreased white matter in the left parahippocampal gyrus in non-demented elderly ε4 carriers compared to non-carriers.

2.6.8 Contradictory findings

While earlier studies reported an association between the presence of the ε4 allele and the risk for Alzheimer's disease in a dose-dependent manner (Corder, et al., 1993; Rebeck, Reiter, Strickland, & Hyman, 1993; Mullan & Crawford, 1993; Saunders, Strittmatter, Schmechel, George-Hyslop, Pericak-Vance, et al., 1993; Strittmatter, et al., 1993), more recent reports make it clear that while the ε4 allele influences the age of onset for those who develop the disease, it is not predictive of caseness nor does it increase lifetime incidence in general (Khachaturian, Corcoran, Mayer, Zandi, & Breitner, 2004). Similarly, a longitudinal study of healthy elderly did not find the ApoE genotype predictive of cognitive decline (Marquis, Moore, Howieson, Sexton, Payami, et al., 2002).

Bassett et al., (2006) presented an analysis of cross-sectional data for 95 asymptomatic offspring (50-75 years of age) of autopsy-confirmed late-onset familial AD cases and 90 age-matched controls, studied with fMRI to investigate brain activation patterns. Analysis of activation in response to a paired-associates memory paradigm found significantly different patterns in these groups. At-risk individuals showed more intense and extensive activation in the frontal and temporal lobes including the hippocampus during memory encoding, an increase unrelated to the ApoE ε4 allele, however, according to these authors. Increasing age and the presence of an affected first-degree relative were the only factors consistently associated with an elevated risk for developing the disease. In addition, there is further evidence that among dementia cases or those with mild cognitive impairment, the ε4 allele has low positive predictive value for the diagnosis of Alzheimer's disease (Devanand, Pelton, Zamora, Liu, Tabert, et al., 2005; Slooter, Breteler, Ott, Van Broeckhoven, & van Duijn, 1996; C. Wang, Wilson, Moore, Mace, Maeda, et al., 2005).

2.6.9 Possible reasons for discrepancy among studies

Various methodological differences may account for the differences among studies. Examples include differences in subject selection, in levels of cognitive performance, in age groups, etc. Differences in cerebral activation studies can be accounted for by various other additional factors; differences between imaging modalities, different methods of analyses, different applied statistical thresholds etc. The proportion of homozygotes and heterozygotes in different studies may also have an important impact on statistical power. More importantly, there are differences in the underlying cognitive functions being tested (i.e. naming fluency, face naming, verbal working memory, verbal episodic memory, nonverbal memory and picture learning) (Scarmeas & Stern, 2006; Bondi, et al., 2005; Smith, et al., 2002; Bookheimer, et al., 2000).

2.6.10 Biological and functional considerations

Effect of the ApoE genotype on lipid metabolism (Srinivasan, Ehnholm, Elkasabany, & Berenson, 2001), blood pressure (Katsuya, Baba, Ishikawa, Mannami, Fu, et al., 2002), atherosclerosis (Hixson, 1991), ischemic heart disease (van Bockxmeer & Mamotte, 1992), myocardial infarction (Brscic, Bergerone, Gagnor, Colajanni, Matullo, et al., 2000), and cognitive performance in type I diabetes (Ferguson, Deary, Perros, Evans, Ellard, et al., 2003) have been documented in very young subjects and in children (Rask-Nissila, Jokinen, Viikari, Tammi, Ronnemaa, et al., 2002; Tammi, Ronnemaa, Rask-Nissila, Miettinen, Gylling, et al., 2001). Animal studies have indicated that ApoE genotype seems to affect stress response and spatial memory (Zhou, Elkins, Howell, Ryan, & Harris, 1998) and regulates synaptic plasticity and long-term potentiation in the hippocampus (Valastro, Ghribi, Poirier, Krzywkowski, & Massicotte, 2001) of young mice. Therefore, it is conceivable that ApoE-related alterations in cerebral physiology may exist from a very young age. There are known early biochemical changes in neuronal processes and synapsis loss before structural pathology is detected (Selkoe, 2002). It is also known that symptoms of AD are preceded by a period of unknown duration during which neuropathological alterations accumulate in the brain without associated memory loss or other detectable cognitive change. The increased risk for AD in subjects carrying the ε4 allele is thought to be mediated by the ApoE genotype being implicated in β amiloyd and/or neurofibrillary tangle biochemical pathways (Polvikoski, Sulkava, Haltia, Kainulainen, Vuorio, et al., 1995; Richey, Siedlak, Smith, & Perry, 1995). Increases and decreases in activations in ε4 carriers have been observed in imaging studies during cognitive tasks [e.g.(Scarmeas, Anderson, Hilton, Park, Habeck, et al., 2004b)]. Overall, the interpretation of increases and decreases in activation has been very controversial. Areas with differential activation in the ε4 carriers may reflect malfunctioning (taking the form of either overactivation or deactivation) because of more severe AD pathological involvement for ε4 carriers in these regions. Alternatively, some of these regions may still be spared by AD pathology and are recruited for task performance by ε4 carriers because of more severe pathological involvement in other regions. Scarmeas et al., (2006), for example, suggested that several other considerations make the possibility of silent AD pathology causing ApoE-related differences less plausible. The detection of ApoE-related differences in resting brain blood flow and activation four to five decades before the possible onset of dementia weakens the hypothesised link with AD. The presence of the ε4 allele is not equivalent to early AD; a significant proportion of ε4 heterozygotes will never develop AD. It is proposed that the presence of ε4 facilitates rather than causes AD (Meyer, et al., 1998), and ε4 has been implicated in impaired brain repair mechanisms that may place subjects at increased risk for AD or other brain diseases.

Therefore, the imaging patterns observed in ε4 carriers may be the early signature of ApoE-dependent alterations in brain physiology, which may result in greater vulnerability to environmental effects such as traumatic brain injury or other insult later in life. To improve the detection of preclinical markers, neuropsychological functioning, brain structure, and brain functioning of at-risk individuals who develop AD should ultimately be tested against that of persons who remain dementia-free over the same follow-up period. Furthermore, these individuals might be compared to those with other conditions i.e. depression or other dementia. In addition to documenting the cognitive deficits in preclinical AD, the longitudinal course of these deficits is also important (Scarmeas & Stern, 2006).

There has been a lack of consensus regarding when the preclinical period begins and how early preclinical changes may be detected. With longer test-retest intervals, cognitive changes are more likely to be detected, but it is more difficult to determine when decline begins. Therefore, the question of the age range, which it is possible to detect a preclinical AD state and whether such states can be distinguished from lower levels of cognitive reserve, should be addressed.

2.6.11 Summary of the chapter

Several levels of evidences have underlined the importance of the ApoE ε4 mutation in predicting AD many years before the clinical onset. Neuroimaging techniques, including functional, structural MRI and different varieties of PET imaging, all show differences between ApoE ε4 carriers and non-carriers in the severity of brain changes in AD, MCI, healthy elderly controls and young individuals. However, to date no studies have attempted to combine data across methodologies to predict cognitive decline in conjunction with genetic risk status.

CHAPTER 3 Neuropsychological changes in preclinical AD

3.1 Introduction

The study of the earliest cognitive markers of a preclinical phase of Alzheimer’s disease has both scientific and clinical importance. From a scientific perspective, research might increase the understanding of the subtle cognitive sequelae that accompany the insidious onset of AD over and above the significant cognitive decline associated with the normal aging process. Clinically, the development of promising pharmacological treatments of AD that may slow or arrest the disease process has created a need to diagnose AD in its earliest preclinical phase, before significant brain damage has occurred. Neuropsychological studies in this area have benefited greatly from the discovery of a genetic risk factor of AD, the allele of the Apolipoprotein E (ApoE ε4) (Corder, et al., 1993), which has enabled neuropsychologists to focus the search for preclinical cognitive markers of AD on samples of normal elderly individuals who are genetically predisposed to develop this disease (Bondi, Salmon, Galasko, Thomas, & Thal, 1999; Elias, Beiser, Wolf, Au, White, et al., 2000).

A few studies have shown no effect of the ApoE ε4 in the rate of cognitive decline (Welsh-Bohmer, Ostbye, Sanders, Pieper, Hayden, et al., 2009; Salo, Ylikoski, Verkkoniemi, Polvikoski, Juva, et al., 2001; G. M. Murphy, Jr., Taylor, Kraemer, Yesavage, & Tinklenberg, 1997). However, many other studies exploring this association have continually shown a deteriorating effect of the ApoE genotype in LOAD with a dose-response manner. In general these studies can be divided into observations including subjects with cognitive impairment or dementia and reports in non-demented individuals, to clarify the effect of this mutation on cognition in people at the preclinical stage.

3.2 ApoE-related cognitive impairment

3.2.1 Clinicophatological studies

It is already well known that AD is a complex disease with multiple genetic and environmental risk factors. To cause cognitive impairment, these risk factors must interact with the types, quantities, and distributions of phatological lesions in the brain. For example, it is likely that some risk factors cause cognitive impairment by enhancing amyloid production or fibrillogenesis, decreasing amyloid clearance, or by enhancing tau phosphorilation. Understanding the neurobiology that links biological factors to cognitive impairment has important implications for disease treatment and prevention.

As for the ApoE ε4 allele, the neurobiological changes in the brain that account for the association of allele status with disease risk remain poorly understood. ApoE is synthesised primarily by astrocytes and microglia in the brain and its main function is thought to be in the regulation of lipoprotein metabolism (Mahley & Rall, 2000). However, preclinical data from a variety of sources suggest that ApoE may also be involved in the deposition and/or clearance of amyloid from the brain. This gene appears to enhance spontaneous fibrillogenesis of amyloid in vitro (Wisniewski, Castano, Golabek, Vogel, & Frangione, 1994), and has been reported to form dimeric complexes with soluble amyloid in vivo (Permanne, Perez, Soto, Frangione, & Wisniewski, 1997). Transgenic mice studies have been done to better clarify the role of ApoE on the deposition of amyloid (Holtzman, Bales, Tenkova, Fagan, Parsadanian, et al., 2000a; Holtzman, et al., 2000b; Bales, Verina, Cummins, Du, Dodel, et al., 1999). Severe neuritic dystrophy was seen in association with amyloid in transgenic mice with mice expressing human ApoE ε4 showing a greater effect on amyloid plaque formation.

In this regard several studies have reported a relationship between the ε4 allele and amyloid deposition (Thaker, McDonagh, Iwatsubo, Lendon, Pickering-Brown, et al., 2003; Ohm, Scharnagl, Marz, & Bohl, 1999), suggesting that amyloid deposition accounts for the association of allele status with cognitive function. Some authors quantified extracellular deposits of amyloid-β peptide plaque deposits and phosphorylated tau immunoreactive neutofibrillary tangles from several regions in healthy subjects and AD, and examined a hypothesised sequence of events linking ApoE allele status to level of cognition assessed proximate to death (Bennett, Schneider, Wilson, Bienias, Berry-Kravis, et al., 2005) (see Figure 3.1.).

APOE ε4 → Amyloid → Tau tangles → Cognitive function

Figure 3.1. *Hypothesised sequence of events that links ApoE allele status to level of cognition proximate to death [Figure taken from (Bennett, et al., 2005)].*

They found that amyloid burden accounted for the association of the ε4 allele to the level of cognitive function. Further, they found that amyloid burden accounted for the association of allele status to tangles, but tangles did not account for the association of the ε4 allele to amyloid burden. Together with data from a prior study (Bennett, Schneider, Wilson, Bienias, & Arnold, 2004), these data suggest that the

ApoE ε4 allele is the first step in a sequence of events that works through amyloid deposition and subsequently tangle formation to cause cognitive impairment.

Understanding how neuropathological indices account for the association of risk factors to cognitive impairment has important implications regarding strategies to delay disease onset. Over the past several years, the presence of one or more ApoE ε4 alleles in the genotypic allele have emerged as the most important genetic susceptibility factor for AD among older people.

Despite intensive investigation, the neurobiological changes responsible for the association of allele status with the occurrence of clinical disease remain controversial, with some data suggesting that its effect is mediated by an increase in the rate of accumulation of AD pathology (Tiraboschi, et al., 2004;Sparks, Scheff, Liu, Landers, Danner, et al., 1996) whereas other data suggest that it may be related to other less specific mechanisms such as neural repair or survival (Berg, McKeel, Miller, Storandt, Rubin, et al., 1998; Pitas, Ji, Weisgraber, & Mahley, 1998).

Many authors have reported that allele status is related to AD pathology as identified with routine histopathology. Further, several studies have found a relation between the ε4 allele and amyloid deposition (McNamara, Gomez-Isla, & Hyman, 1998; Gearing, Mori, & Mirra, 1996) or both amyloid and tau positive tangles (Thaker, et al., 2003; Ohm, et al., 1999). By contrast, some studies have found an association of the ε4 allele and amyloid deposition but not tau tangles (Mukaetova-Ladinska, Harrington, Roth, & Wischik, 1997). These data show an association of allele status with amyloid deposition and tangles formation, but also provide strong evidence that amyloid deposition mediates the association of the ε4 allele with tau tangles and ultimately with the level of cognitive function. More specifically, accounting for amyloid burden in the analyses remarkably attenuates the association of allele status with cognitive function. This finding does not preclude the possibility that the ε4 allele may work through other mechanisms in addition to amyloid, but it implies that the effect size of the other variables would be substantially smaller.

Other interesting studies underline the role of Butyrycholinesterase (BuChE) in the CNS and its involvement in the pathology of AD (Darreh-Shori, et al., 2006). Their data support a strong link between the levels of BuChE expression and the progress of this neurodegenerative disorder. These authors found that a higher level of CSF BuChE activity or protein level in the patients was associated with better cognitive performance in several neuropsychological tests such as global cognition (MMSE and ADAS-cog), episodic memory and attention compared to the AD patients with a low CSF BuChE activity. These observations support previous finding that BuChE might play an important role in regulating some cognitive functions mediated by cholinergic or related neuronal networks in the brain (Darvesh & Hopkins, 2003; Mesulam, 2000). These authors also found that CSF BuChE activity and protein levels differed in AD patients that were carriers or non-carriers for the ApoE ε4 allele (ε4 -/- > ε4+/- > ε4 +/+). The patients with low CSF BuChE activity were exclusively carriers of one or two ApoE ε4 alleles.

These patients in general had 20-26% less BuChE protein and 40-60% less activity in the CSF BuChE than did the ε4 non-carriers. In addition, they observed a gender-related difference in BuChE levels, i.e. a lower level of CSF BuChE in female AD patients than in males. Intriguingly, most epidemiological studies report female gender as one of the risk factors for AD (Fratiglioni, et al., 2000).

Others physiopathological studies describe the relationship between tau markers in the cerebrospinal fluid (CSF), the degree of cognitive impairment and the predictive value of epigenetic markers such as carrying the ApoE ε4 allele, as part of a longitudinal study. The findings indicate that hyperphosphorylated tau is a good indicator of the degree of cognitive disorders in the early stages of AD and that no clear correlation exists with the ε4/ε4 and ε4/ε3 genotypes, even though a higher proportion of carriers of the ε4 allele was present in the MCI group with a more significant level of impairment and in AD patients.

3.2.2 Neuropsychological studies across the life span

Recent studies indicate that ApoE ε4 might affect the rate of cognitive decline in the early and late stages of AD. In a large sample of 56 year old patients with LOAD, the presence of at least one ε4 allele was associated with faster cognitive decline most significantly in the earliest stage of AD (p= 0.01) (Cosentino, Scarmeas, Helzner, Glymour, Brandt, et al., 2008). Wehling et al., (2007) in a study with 70 patients (50-75 age range) found poorer performance in MMSE and California Verbal Learning Test (CVLT) in ε4 carriers than non-carriers. Hirono et al., (2003) examining the effect of ApoE on cognition using the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) in 64 LOAD patients found that the presence of the ε4 allele affected memory performance in a dose dependent manner. Compared with older adults who remained non-demented, those who later develop AD performed more poorly across a broad range of neuropsychological measures.

One critical review about changes in preclinical AD presents a summary of 73 studies of neuropsychological changes in the preclinical period (Twamley, et al., 2006). There were 30 longitudinal case-control studies; 16 longitudinal studies examining decline in ApoE ε4 + and ε4– subjects; 26 cross-sectional studies comparing neuropsychological performance in subjects with and without the ApoE ε4 allele; three retrospective studies using autopsy data and four studies comparing neuropsychological performance in subjects with and without a family history of AD. The domains most consistently associated with preclinical AD were attention, verbal learning and memory, executive functioning, processing speed and language, with studies showing either early decline in these abilities or significant differences between at-risk subjects and control subjects. This review revealed that attention, although not as commonly assessed as learning and memory in studies of preclinical AD, is even more consistently associated with later development of AD. Furthermore, verbal learning was a somewhat more consistent indicator of preclinical AD than verbal delayed recall. These findings suggest that the deficits in verbal delayed recall in preclinical AD may partly reflect poor attention at encoding. Baxter et al., (2003) also found that verbal learning ability declined over two years in a group of cognitively normal individuals who had the ε4 allele, but only in those who were 60 years of age and older.

Episodic memory decline is one of the earliest and most prominent features of preclinical AD (Bondi, et al., 1999; Bondi, Salmon, Monsch, Galasko, Butters, et al., 1995). Using the California Verbal Learning Test (CVLT) in 52 non–demented elderly (14 carriers and 26 non-carriers), Bondi et al., (1995) found that ApoE epsilon 4 subjects demonstrated significantly poorer mean performances than non-epsilon 4 subjects on nine CVLT variables. Six of the carriers who completed an annual follow up assessment developed LOAD. None of the 26 non-carriers showed any significant cognitive decline. Subtle decline in episodic memory often occurs several years before the emergence of the obvious cognitive and behavioural changes required for a clinical diagnosis of AD (Albert, Moss, Tanzi, & Jones, 2001; Lange, et al., 2002).

Several longitudinal studies confirmed strong association between the presence of the ApoE ε4 allele and poorer episodic semantic performance when compared with non-carriers over a 8 year follow up period (Mayeux, Small, Tang, Tycko, & Stern, 2001; Wilson, Schneider, Barnes, Beckett, Aggarwal, et al., 2002). Further more recent studies found similarly strong association in the rate of change in episodic memory among ApoE ε4 carriers (Lehmann, Refsum, Nurk, Warden, Tell, et al., 2006). It is thought that episodic memory tasks are strong predictors of future AD, because the brain structures sub-serving episodic memory, such as medial temporal lobes and the hippocampal formation, are amongst the first affected.

Studies of association between ApoE ε4 and working memory (WM) have also been carried out. WM is a cognitive system which permits temporary storage of the information in order to keep it in an active state to be manipulated and protected from interference. Several studies investigating Stroop performance in AD patients have demonstrated deficits on the traditional interference condition and this impairment tends to be present early in the course of the disease (Bondi, Serody, Chan, Eberson-Shumate, Delis, et al., 2002). Deficits on other tasks of executive functioning have also been found in AD patients (Johnstone, Hogg, Schopp, Kapila, & Edwards, 2002).

Recent evidence suggests that such deficits may also be present in a preclinical phase of AD. Rosen et al., (2002) using the Operation Span task which emphasize maintaining information in storage while performing distracting operations found that the operation span was more negatively affected in ε4 carriers than in non-carriers in healthy normal subjects with a mean age of 62 years. In another study Wishart et al., (2006) found greater fronto-temporal activation in ε3ε4 carriers in an N-back task than ε3 homozygotes despite equal behavioural performance, indicating that more effort reflected in greater activation could underline intact performance.

In a retrospective study, Albert et al., (2001) found that tests of set shifting [Trail Making Test, Part B; (Reitan, 1955)] and sequencing [Self-Ordering Test; (Petrides & Milner, 1982)] were predictive of onset of AD. Chen et al., (2000) carried out a retrospective study of individuals who later developed AD and also found that deficits on Part B of the Trail Making Test predicted AD onset in this group. In addition, these findings suggest that subtle deficits in executive functioning may signal a preclinical phase of AD. Although a number of variants of the Stroop Test have been developed over the past 70 years, a new version of this task (The D-KEFS Color-Word Interference Test; CWIT) includes the three traditional stroop conditions, and adds a fourth condition called Inhibition/Switching (Delis, Kaplan, E., & Kramer, J.H., 2001). The stimuli in this new condition are the same as in the traditional interference condition with the exception that half of the words are printed inside a small box. This condition requires the examinee to name the dissonant ink colour for a word not in a box, and to switch set and read the printed word (rather than name with dissonant ink colour) for items within a box. A prospective study of non-demented older adults at genetic risk for AD (i.e. ApoE ε4 allele) and other types of dementia utilized this new Stroop Task that included a dual executive-function condition requiring both response inhibition and cognitive switching (Wetter, Delis, Houston, Jacobson, Lansing, et al., 2005). This study found that, relative to the non-ε4 subjects, the ApoE ε4 subjects made significantly more errors on the CWIT, but only on the new Inhibition/Switching condition of the test. Thus, the ε4 group's poor performance on the Inhibition/Switching condition is likely not due to difficulties with reading speed, naming speed or response inhibition per se. As the Inhibition/Switching condition requires an individual to simultaneously engage in response inhibition and cognitive switching, these findings suggest that the higher demands of this task resulted in a subtle deficit in the ApoE ε4 group. It may be the case, therefore, that the traditional Stroop task is sufficiently sensitive to identify deficits in individuals who have already received an AD diagnosis, but the new Inhibition/Switching condition, which assesses both response inhibition and cognition shifting, may be required to identify subtle deficits in healthy individuals at risk for AD. Recently, Reinvang et al., (2010) found a negative effect of the ApoE ε4 carriers in a dose-related way on working memory measured with different tasks focused on goal maintenance, storage capacity and interference control. Although it may not rule out the possibility that the increased load of AD pathology (accumulation of β amyloid in the brain after age 60) seen in healthy older ε4 carriers (Reiman, et al., 2009; Small, Siddarth, Burggren, Kepe, Ercoli, et al., 2009) could explain these results, it cannot also rule out an influence of ApoE ε4 on accelerated aging effects on working memory. In support of this last hypothesis there is evidence of ApoE ε4 effects on cognitive and brain function even in young adults (20-40 years old). It is unlikely, at this young age, that this cognitive phenotype could be due to the very early Alzheimer's disease relates pathological changes (Scarmeas, et al., 2005a; Reiman, et al., 2004).

As described previously, ApoE ε4 is a strong predictor of progression to AD in MCI patients (Petersen, Smith, Ivnik, Tangalos, Schaid, et al., 1995). Moreover, greater medial temporal lobe atrophy in AD patients carrying the ApoE ε4 allele has also been documented (Lehtovirta, Laakso, Frisoni, & Soininen, 2000; C. R. Jack, Jr., et al., 1998b). Interestingly, Negash, et al., (2007) investigated effects of the ApoE genotype and MCI on implicit learning using two different paradigms sequence, learning and contextual cueing, which rely on two different neuronal systems. Neuroimaging studies of healthy adults and brain injured patients showed that learning of sequences is mediated by a fronto-striatal-cerebellar system (Willingham, Salidis, & Gabrieli, 2002; Gomez Beldarrain, Grafman, Pascual-Leone, & Garcia-Monco, 1999); whilst contextual cueing learning depends on medial temporal lobe structures (Chun & Phelps, 1999; Manns & Squire, 2001). Negash et al., (2007) wanted to determine the extent to which these two forms are influenced by ApoE genotype in MCI and healthy controls. These authors observed that healthy elderly controls carrying the ApoE ε4 allele showed contextual cueing deficits compared to those who did not carry the ApoE ε4 allele, while by contrast, sequence learning appeared not to be influenced by ApoE genotype. Interestingly, control carriers revealed similar performance in contextual learning as the MCI group, while the non-carriers performed better. These behavioural data support findings of medial temporal dysfunction and relative integrity of fronto-striatal system in MCI. Furthermore, this study suggests the possible vulnerability of ε4 carrier healthy adults on tasks relying on medial temporal structures compare to non-carriers. Moreover Hsiung et al., (2004) found an increased risk of conversion from MCI to LOAD and decreased age of onset of LOAD in patients carrying at least one ε4 allele.

In a recent retrospective study, Caselli et al., (2007) showed that ApoE ε4 homozygotes in their 60s had higher rates of cognitive decline than ApoE ε4 heterozygotes or non-carriers before the diagnosis of MCI and AD, thus confirming and characterising the existence of a pre-MCI state in this genetic subset. Recently Boyle et al., (2010) in a longitudinally study (with a 16 year follow up) found that the presence of the ApoE ε4 allele was associated with a 1.4 fold increased risk of incipient MCI. Moreover this mutation was associated with an increased rate of decline in episodic memory, semantic memory, working memory and perceptual speed. Tupler et al., (2007), in a longitudinal genetic analysis showed that performance on the CVLT recall after a delay interval was most strongly accounted for by the score on the task obtained 5 years early, followed by carrying the ApoE ε4 alleles, followed by left hippocampal volume. These findings are supported by another study which shows the significance of ApoE ε4 as a predictor of conversion to AD in forward regression models examining different variables form the CVLT and Wechsler Memory Scale (Lange, et al., 2002). Interesting, there are also longitudinal studies from twins showing significant low performance during recall memory in ε4 carriers compared to e4 non-carriers (Reynolds, et al., 2006; Schultz, Lyons, Franz, Grant, Boake, et al., 2008).

Another interesting aspect came from an analysis of an ApoE gene-cognitive decline interaction. Several studies showed that age and ApoE ε4 allele-dose interact contributing to the presence and magnitude of ApoE ε4 related deficits. As for dose-related deficits, several studies show greater cognitive impairment in ε4 homozygous old healthy subjects whilst the opposite pattern seems to characterise performance in young individuals. In a prospective cohort study, Nillson et al., (2006) showed that among old participants ε4 homozygous performed the worst and non-carriers obtained the best in episodic memory and recall performance. However, among young subjects, superior performance of ε4 homozygous carriers was found. Consistent results were also seen in similar studies (Schultz, et al., 2008; Reynolds, et al., 2006; Riley, et al., 2000; B. J. Small, et al., 2004). This pattern of results also mirrors the relationship between ApoE ε4 and risk of AD with advanced age decreasing the influence of this mutation in predicting AD (Breitner, Wyse, Anthony, Welsh-Bohmer, Steffens, et al., 1999; Farrer, et al., 1997). Of interest in this respect are the findings of studies which examined the relationship between ApoE genotype and cognitive performance among children. Turic et al., (2001) found no significant

ApoE ε4 related differences in children when they looked at general cognitive ability scores. Similarly, Deary et al., (2003) found no significant interaction between ApoE ε4 and general intelligence scores in 11 year olds, although ε4 carriers had lower scores. Wright et al., (2003) showed that ε4 carrier infants performed higher than ε3 or ε2 carriers in the 24 month-Bayles Scale infant Development Score, suggesting an advantage for carriers with respect to early-life neuronal/brain development. Finally, Oria et al., (2005) showed an advantageous effect of the ApoE ε4 allele in semantic fluency in children with heavy burdens diarrhea and instead profoundly impaired semantic fluency in ApoE ε4 non-carrier children. These results suggest that maybe a gene may have a different function at different stages in life. In particular positive effects early in life and deleterious effects in older age, a phenomenon called antagonist pleiotropy (Williams, 1957) (see section 2.6.3.). The relative magnitude of the findings suggest that premorbid ability or background cognitive reserve, genetic contribution of ApoE ε4 load and structural brain characteristics, are the predominant factors in predicting performance over time.

3.2.3 Linguistic changes in verbal expression: A preclinical marker of Alzheimer's disease

A few studies have examined the presence of linguistic deficits in the preclinical phase of Azheimer's disease. This phase is known to be characterised by the presence of subtle cognitive impairment, which appears decades before a clinical diagnosis of probable AD can be made. Elias and colleagues (2000) reported that cognitive changes have even been detected 20 years before clinical diagnosis. As described in the previous sections, alterations in various cognitive functions, including episodic memory (Lehmann, et al., 2006), executive functions (Reinvang, et al., 2010), attention (Rapp & Reischies, 2005 ;Estevez-Gonzalez, Kulisevsky, Boltes, Otermin, & Garcia-Sanchez, 2003), spatial-visual abilities (Small, Herlitz, Fratiglioni, Almkvist, & Backman, 1997b) and psychomotor speed (Masur, Sliwinski, Lipton, Blau, & Crystal, 1994) have been reported in the preclinical phase of AD.

Research examining linguistic changes in patients in the minimal to mild stages of AD reports that semantic processes are often found to be affected (e.g. patients simplify grammatical structure, ineffectively communicate information, fail to identify pictorial themes, and lose vocabulary; (Forbes-McKay, Shanks, & Venneri, 2004; Garrard, Maloney, Hodges, & Patterson, 2005; Forbes-McKay, Venneri, A., & Ellis, A.W. , 2003; Forbes-McKay, Venneri, & Shanks, 2002).

There is less consensus in the literature regarding the presence of linguistic changes in the preclinical phase of AD. The few research studies that have been carried out with individuals in the preclinical stage of AD have produced contradictory findings. Although four early studies found no changes in linguistic abilities in the preclinical stage of AD (e.g. Almkvist, 1995; Farlow, Murrell, Ghetti, Unverzagt, Zeldenrust, et al., 1994; Newman, Warrington, Kennedy, & Rossor, 1994; Karlinsky, Vaula, Haines, Ridgley, Bergeron, et al., 1992), other more recent findings support the hypothesis that linguistic changes are evident before the clinical onset of AD. In a case study, a linguistic analysis of Iris Murdoch's final novel, published one year before the diagnosis of probable AD was made, showed deterioration of semantic skills and sophistication of vocabulary (Garrard, et al., 2005). In a sub-analysis of 93 participants in the Nun study, a longitudinal study of aging and Alzheimer's disease, Snowdon et al., (1996) reviewed the early-life autobiographies in order to investigate the relationship between linguistic abilities in early life and cognitive function and neuropathologically confirmed AD in late life. "Low idea density” in the autobiographies was significantly associated with an increased risk of poor cognitive function and Alzheimer’s disease 58 years later. Similar findings were obtained from analysis of the public speeches by former U.S. president Ronald Reagan, who was diagnosed with probable AD in 1994 (Venneri, Tumbull, O., & Della Sala, S. , 1996). These analyses show that word finding difficulties and inappropriate phrases were apparent in 1981, 13 years before his diagnosis.

Other epidemiological studies provide additional evidence for linguistic changes in the preclinical stage of AD. Jacobs et al., (1995a) administered a comprehensive neuropsychological battery to a group of initially non-demented older adults participating in the North Manhattan Aging Project, a prospective epidemiological study of dementia. A total of 41 of the 443 participants were diagnosed with incident, probable or possible AD at one of the 1-to 4-year follow-ups. In this study, baseline scores on the Boston naming Test were found to predict subsequent AD diagnosis.

In a research study of naming of famous faces abilities in individuals with and without -MCI- who would or would not go on to develop dementia of the Alzheimer’s type (DAT) and a control group, Estevez-Gonzalez et al., (2004) found that those individuals with MCI who were diagnosed with DAT two years later performed significantly worse on this task in the preclinical phase than did those with MCI who did not develop DAT or control participants.

In a population-based study in Sweden which examined whether cognitive variables at baseline could predict incident cases of AD after a 3-year follow-up, Small et al., (1997) found that recognition of faces and letter fluency were reliable predictors of dementia status, independently of the Mini-Mental State Examination (MMSE) score. Using data from a Swedish population-based study, Jones and colleagues (2006) confirmed that letter fluency performance was indeed significantly worse 3 years before AD diagnosis, in older adults developing the disease than in healthy controls who did not develop AD. Furthermore, these researchers found that category fluency performance was significantly worse for those who will develop AD than for controls. It is well known that a category fluency task, which requires naming words belonging to a particular category, puts strong demands on the hierarchical structure of semantic knowledge (Butters, Granholm, Salmon, Grant, & Wolfe, 1987) . Letter fluency, which requires naming words that begin with a specific letter, is thought to rely on more "frontal control" regions (Delis D, 2001; Bryan, Luszcz, & Crawford, 1997). Several studies have shown greater semantic fluency impairments in AD patients than a letter fluency tasks (Chan, Butters, Salmon, & McGuire, 1993). Investigation of the discriminative value of fluency subtypes in AD showed that semantic fluency (100% sensitivity, 92.5% specificity) is superior to letter fluency (89% sensitivity, 85% specificity) in predicting group membership (Monsch, Bondi, Butters, Salmon, Katzman, et al., 1992). Saxton et al., (2004) showed that the category fluency task was one of the best predictors of subsequent conversion to AD in older adults. These behavioural findings are also supported by lesion studies and brain imaging research in AD (Mungas, Jagust, Reed, Kramer, Weiner, et al., 2001; Gourovitch, Kirkby, Goldberg, Weinberger, Gold, et al., 2000; Paulesu, Goldacre, Scifo, Cappa, Gilardi, et al., 1997). Henry et al., (2004) examined a focal lesion cohort and showed that the letter fluency task is more sensitive to frontal lobe lesion whilst the category fluency task is more sensitive to temporal lobe lesions. fMRI with young adults whilst doing a category fluency task activated hippocampal complex in all subjects (Pihlajamaki, Tanila, Hanninen, Kononen, Laakso, et al., 2000). In general, both letter and category fluency tasks depend on a common network of brain areas but the degree of activation appears to be greater in the MTL in the category fluency task and in frontal areas in the letter fluency task. Although these studies support the idea of an increased difficulty in semantic relative to letter fluency tasks in AD and showed a strong association of this task with the MTL, a region which is affected at very early stage by AD neuropathological decline, it is unclear if this is the case in the early preclinical stage.

In general it has been observed that verbal fluency discriminates accurately between MCI subjects and healthy controls (Tabert, Manly, Liu, Pelton, Rosenblum, et al., 2006; Geslani, Tierney, Herrmann, & Szalai, 2005; Grundman, Petersen, Ferris, Thomas, Aisen, Bennett, Foster, Jack, Galasko, Doody, Kaye, Sano, Mohs, Gauthier, Kim, Jin, Schultz, Schafer, Mulnard, van Dyck, Mintzer, Zamrini, Cahn-Weiner, & Thal, 2004; Bennett, Wilson, Schneider, Evans, Beckett, et al., 2002). One of the questions arising from these studies is the utility of using different semantic categories to elicit different results. Standish et al., (Standish, Molloy, Cunje, & Lewis, 2007)showed no differences in performance on four different categories in MCI groups. The overall score discriminated this group from healthy elderly controls. Murphy et al.,(2006)found that amnestic MCI and AD produced fewer words in the semantic fluency task than controls, with relative preservation of performance of the letter fluency task in the amnestic MCI group. Vogel et al., (2005) studied 22 individuals in the preclinical stage and 58 healthy controls and found significant differences on category fluency between individuals with slight cognitive impairment, but without dementia, and controls. Jones et al., (2006) studied 66 individuals in the preclinical stage of AD and 267 healthy controls and found that category fluency was impaired in individuals who developed AD one to three years later. Adlam et al., (Adlam, Bozeat, Arnold, Watson, & Hodges, 2006) investigating semantic memory in mild AD and MCI showed a widespread impairment on all the semantic tasks used in the study with greater impairments in the MCI group. In contrast, the MCI group did not differ significantly from controls except on category fluency. Finally, Murphy and colleagues (Murphy, Rich, & Troyer, 2006) showed a similar trend in the pattern of performance in the category fluency task among controls, aMCI and AD. Category fluency, therefore, appears to be affected in the preclinical stage of AD.

However other studies have found that category fluency tasks are not sensitive to the decline in MCI, although MCI showed a trend toward that direction (Karrasch, Sinerva, Gronholm, Rinne, & Laine, 2005). The reasons for these contradictory findings could be ascribed to the difficulty to make a correct diagnosis of MCI or to include different MCI typologies.

In summary, in the preclinical phase of AD, linguistic tasks that rely on semantic processes, including naming of line drawings, spontaneous writing, naming of famous people, and verbal fluency, appear to be the most vulnerable to the early effect of AD neuropathology. In contrast, tasks that use sub-lexical pathways, such as reading aloud, lexical decision, and repetition of words, seem to be relatively well preserved until the most advanced stages of the disease appear (Arango Lasprilla, Iglesias, & Lopera, 2003).

Interestingly some authors examined finer aspects of verbal fluency tasks. Forbes and colleagues (2005) investigated the qualitative lexical characteristics of the individual items produced in the category fluency task by controls and minimal to moderate AD patients. They found that the age of acquisition (AoA) of items produced differentiated healthy controls from AD although this value wasn't a good predictor of AD severity. Holmes and colleagues (2006) found that AD patients failed to classify more late than early acquired objects as real compared to a control group, showing a differential impairment in naming late acquired objects. These findings are consistent with other studies which indicate that AoA is an important factor in naming performance in AD (Funnell, 2005; Silveri, Cappa, Mariotti, & Puopolo, 2002;Kremin, Perrier, De Wilde, Dordain, Le Bayon, et al., 2001). The analyses of these subtle changes may benefit the preclinical diagnosis of AD and might be useful in the development of cognitive interventions to maintain linguistic functioning or in the prevention of decline in patients at risk of developing AD.

The existence of a group of people who are healthy carriers of a genetic mutation that has been likely identified at increasing risk of developing AD provides a unique opportunity to examine the appearance of symptoms in the preclinical phase of the disease, because these individuals will develop AD with a higher possibility than non-carriers. By comparing carriers of a genetic mutation for AD who do not yet have clinical symptoms of the disease to healthy non-carrier family members, it is possible to determine whether linguistic deterioration does indeed occur before the clinical phase starts and, if deterioration is found, this finding will provide an opportunity to elucidate which aspects of verbal expression are the first to deteriorate.

A few studies have been published examining linguistic changes in the preclinical stage of familial AD. Some were descriptive case studies (Farlow, et al., 1994; Newman, et al., 1994) which did not evaluate semantic-lexical processes or had a small sample sizes (Almkvist, Axelman, Basun, Wahlund, & Lannfelt, 2002). Recently a study proposed that semantic changes, as identified by alterations in the naming of famous faces task and in a description of the Cookie Theft Picture Card of the Boston Diagnostic Aphasia Examination, are present in healthy individual carriers of the E280A autosomial dominant mutation in the Presenil-1 gene on chromosome 14, who in the future will develop Alzheimer’s disease (Arango-Lasprilla, Cuetos, Valencia, Uribe, & Lopera, 2007). It is, therefore, possible to detect semantic deterioration in individuals several years prior to the clinical diagnosis of probable familial AD using semantic measures, and early detection of these changes may also be possible in sporadic AD as well.

It is not clear for these studies why lexical semantic abilities should be so vulnerable to the effect of neurodegeneration due to AD. One possibility might be the strong association of these abilities with a normal substrate (mediotemporal cortex and adjacent regions) which is affected by neuropathological changes very early in the course of the disease (Venneri, McGeown, Hietanen, Guerrini, Ellis, et al., 2008). It might also be that semantic abilities are refined and perfected over a long period of time and are represented across a wide network of normal structures (Vandenberghe, Price, Wise, Josephs, & Frackowiak, 1996). It might be, therefore, that development of mature semantic representations might depend on the integrity of a number of factors (i.e. environment, education, genetic profile). In this regard it might also be that ApoE ε4 could act as a stressor (first hit) preventing the development of mature semantic representations through the latent dysfunctional upregulation of tau and AβPP in areas responsible for development of semantic skills (see LEARn model, chapter 1 section 1.7.1.). As a consequence, these data provide a helpful background to build up useful endophenotypes which might provide more informative outcome for understanding the genetics of Alzheimer's disease associated with structural and functional brain changes and the cognitive reserve mechanisms preventing individuals from developing cognitive impairment.

3.3 Aims and Objectives

3.3.1 Introduction

Evidence from studies of language abilities in early AD (Croisile, Ska, Brabant, Duchene, Lepage, et al., 1996; K. E. Forbes-McKay, et al., 2005; K. E. Forbes-McKay, et al., 2004; K. E. Forbes-McKay, et al., 2002; Pestell, Shanks, Warrington, & Venneri, 2000), in people at increasing genetic risk of AD (Arango-Lasprilla, et al., 2007), retrospective language studies (Garrard, et al., 2005; Snowdon, Greiner, & Markesbery, 2000; Snowdon, et al., 1996) and prospective reports of preclinical semantic deficits in AD (Adlam, et al., 2006; Duong, Whitehead, Hanratty, & Chertkow, 2006; Marczinski & Kertesz, 2006) show that deterioration of semantic fluency (and semantic ability in general) seems, therefore, not only to distinguish normal from pathological age related decline, but may offer a useful prognostic indicator in mild cognitive impairment and high risk subjects.

Impairment in semantic tasks in this population might indicate either that individuals who will develop AD never fully developed sophisticated semantic skills, or that the neuroanatomical substrate of semantic abilities is selectively sensitive to the earliest effects of AD neuropathology. The distributed nature of such semantic representations implies that any deficit in development may be so subtle that only sophisticated analysis of linguistic abilities in individuals at risk will detect them.

Taken together, this research indicates that subtle aspects of semantic processing may be deficient in non-demented individuals with the ε4 allele and may serve as an early marker of AD, in the absence of deficits in more global measures of cognition. It is worth emphaising that most research in this area has produced inconsistent findings and has not succeeded in identifying cognitive endophenotypes, perhaps because standard neuropsychological tests were used to assess the level of cognitive abilities. It is reasonable to hypothesise that those tests might not be sensitive enough to detect cognitive differences as a function of ApoE status.

Only a few studies have been carried out to assess the sensitivity of these lexical semantic deficits (age of acquisition, typicality etc) in discriminating AD patients from controls (Venneri, et al., 2005) and no specific studies addressing this issue have been carried out in the preclinical stage of the disease. Moreover, the relationship between the presence of the ε4 allele in the genetic profile and deficits in specific semantic skills, investigated through an analysis of lexical semantic attributes of words produced in a fluency semantic task, has never been tested previously.

The aim of this dissertation is to search for markers of language deterioration which might flag up the presence of abnormal brain ageing and to look for any potential interaction between genetic variation, language deterioration and abnormal brain ageing. Thus, it is worthwhile studying the relationship between the presence of this genetic mutation in the genotype of different groups (high risk and low risk AD and MCI individuals) and the functional spread of cognitive decline, to clarify whether and to what extent ApoE ε4 affects brain atrophy in these groups and to verify if lexical semantic deficits are also sensitive in discriminating preclinical populations at risk for AD. Furthermore, in order to document in the future the possible progressive nature of these lexical deficits in healthy people carriers of the ApoE ε4 allele, a secondary aim of this study was to build up a set of age and education based norms for age of acquisition, typicality and familiarity for items commonly produced on category fluency tasks.

The objectives of this study are:

1. **TO INVESTIGATE THE INTERACTION BETWEEN SEMANTIC AND GENETIC MEASURES IN AD AND MCI.**

   Chapter 4 will focus on detecting possible differences in cognitive decline related to the presence of the ApoE ε4 allele in two small MCI and AD groups. Moreover, a category fluency task (animal and fruit categories) will be administrated and lexical semantic values will be calculated to test whether there is any statistically useful association or at least a trend in this direction between genotypic profile and abnormal lexical-semantic performance. Furthermore, this relationship will be further investigated in a bigger MCI sample.

2. **TO INVESTIGATE THE NEUROANATOMICAL SUBSTRATE OF LEXICAL SEMANTIC DECLINE IN MCI CARRIERS AND NON-CARRIERS.**

   In the first part of Chapter 5 a voxel morphometry study in a group of MCI and mild AD patients will be carried out to better clarify the relationship between ApoE ε4 status and regional grey matter volume. In particular to check if there is a similar ApoE ε4 related atrophy pattern across these two groups to confirm structural brain changes as a possible preclinical endophenotype, when associated with the presence of the ApoE ε4.

   The second study will focus on the interplay between genetic factors, brain atrophy and semantic-lexical deficits in MCI subjects. The aim is to investigate whether atrophy in medial temporal regions, which correlates strongly with age of acquisition and typicality effects and appears to discriminate normal from abnormal cognitive decline, is also associated with the poor lexical semantic performance seen in amnestic MCI carriers of the ApoE ε4 mutation.

3. TO GENERATE NORMATIVE DATA FOR LEXICAL SEMANTIC ATTRIBUTES (AOA, FAMILIARITY AND TYPICALITY).

In order to generate normative data, age of acquisition, typicality and familiarity ratings will be collected from a large sample of healthy individuals between 20 and 95 years of age, who vary in terms of education and sex. The results of this study will be presented in Chapter 6.

CHAPTER 4 Studying the relationship between ApoE status and lexical effects in mild Alzheimer's disease and Mild Cognitive Impairment.

4.1 Linking a genetic risk factor with cognitive predictors

As reviewed in the earlier chapters, to this day, the aetiology of Alzheimer's disease (AD) remains broadly unknown and its natural history is heterogeneous across patients. At the genetic level, mutations of three genes have been identified as linked to early-onset familial AD; amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2), but the inheritance of the disease as a mendelian trait arises in only 2% of all cases (Pericak-Vance, Grubber, Bailey, Hedges, West, et al., 2000). In sporadic AD genetic factors make a significant contribution to aetiology, but are not the main determining factor. Most forms of AD have a complex aetiology and the putative environmental and genetic factors which contribute to causation appear to be necessary but not independently sufficient for the development of the disease. The apolipoprotein E (ApoE) ε4 gene on chromosome 19 has been identified as a major risk factor for sporadic late-onset cases of AD. In established AD, the ApoE ε4 mutation is present in up to 50% to 60% of patients. Compared to those with no copies of the ε4 allele, individuals with one copy of this allele have a three to four times higher risk of developing AD. Two copies of the ε4 allele mean a tenfold increase in the risk of developing the disease (Corder, et al., 1993). AD related ApoE variants consistently correlate with neurophysiological features, neurocognitive functions and biological markers including serum B-amyloid and ApoE level, lymphocyte apoptosis, brain bioelectrical activity, memory function, cerebrovascular hemodynamics, blood pressure, cholesterol level (Cacabelos, et al., 2003a).

It seems reasonable, therefore, to look for possible endophenotypes in currently asymptomatic individuals whose genetic profiles may indicate a hereditary risk for developing AD. The search for cognitive markers has mainly focused on the presence of memory changes, a common early complaint of people who later develop AD (Backman, Small, & Fratiglioni, 2001). In recent years, however, research has shown that language also begins to deteriorate before the disease is manifested and diagnosed (Garrard, et al., 2005; Snowdon, et al., 1996). Some of the linguistic changes found in the early phase of AD include confrontation naming and verbal fluency problems (Alberca, Salas, Perez-Gil, Lozano, & Gil-Neciga, 1999; Appell, Kertesz, & Fisman, 1982; Bayles, Kaszniak, & Tomoeda, 1987; Bayles & Tomoeda, 1983; Huff, Corkin, & Growdon, 1986), loss of vocabulary (Forbes-McKay, et al., 2004; Forbes-McKay & Venneri, 2003; Forbes-McKay, et al., 2002) and difficulty with the naming of famous people (Semenza, Borgo, Mondini, Pasini, & Sgaramella, 2000). In this regard AD patients seem to show more impairment to name unique entities such as famous people rather than objects and this pattern has been seen in predementia people and the amnestic subtype of mild cognitive impairment (aMCI) (Ahmed, Arnold, Thompson, Graham, & Hodges, 2008;Joubert, Felician, Barbeau, Didic, Poncet, et al., 2008), (P. Thompson, et al., 2002).

Variants of the semantic fluency task have frequently been used to characterise the earliest linguistic alterations in AD. Whilst simple and brief to administer, semantic fluency tasks generate potentially rich and salient data. Apart from distinguishing control from patient performance (as controls consistently generate significantly more words than patients), category and letter fluency tasks have also been used to discriminate between patients with semantic dementia, primary progressive aphasia and AD (Marczinski & Kertesz, 2006). More detailed analysis of the characteristics of the words produced in a semantic fluency task may allow more sophisticated dissociations between patients with AD and healthy controls to emerge. For example, the study of spontaneous word retrieval in a semantic fluency task has shown that the lexical effect characterising word production in patients with AD might be a useful predictor of the disease. A longitudinal study carried out by Amieva et al., (2008) showed category fluency impairment about 12 years before the patients fulfiled AD diagnostic criteria clinically. Naming and spontaneous speech experiments have shown that patients with AD have better preserved retrieval of words that are acquired earlier in life, that refer to items which are easier to imagine and that are more typical exemplars of their category (Kremin, Perrier, De Wilde, Dordain, Le Bayon, et al., 2001; Silveri, Cappa, Mariotti, & Puopolo, 2002; Silveri, et al., 2002; Kremin, et al., 2001). Similar results were found by Forbes-McKay et al., (2005). These authors showed that the words generated by AD patients tend to be shorter, earlier acquired, with higher frequency and more typical of the semantic category than the words generated by healthy controls. Each of these characteristics could be used to predict group membership to some degree, but the word attribute that was most successful in distinguishing patients from controls was age of acquisition. Used alone, this lexical factor was substantially better at discriminating patients from controls than number of words produced. Again it could be that age of acquisition task involves a more unique pattern of semantic knowledge than the more general one represented by the other semantic lexical domains like typicality, frequency or length and that this aspect of semantics might be more vulnerable to the early change due to AD.

Other studies examining verbal fluency in the preclinical phase of AD have found significant deficits in category fluency (Clark, Gatz, Zheng, Chen, McCleary, et al., 2009;Jones, et al., 2006; Vogel, et al., 2005). In particular these deficits seem to characterise individuals with amnestic mild cognitive impairment who will develop AD in later life (Adlam, et al., 2006; K. J. Murphy, et al., 2006), giving support to the sensitivity of category fluency for detecting early sign of significant impairment (Hodges, Erzinclioglu, & Patterson, 2006). However, because of the heterogeneity of the MCI population and the data contamination due to the presence in the sample of MCI who will never develop the disease, a better strategy to increase homogeneity in the sample might be the identification of a genetic mutation that confers a significantly increased risk for developing AD. As previously discussed, carriers of genetic mutations which increase the risk of developing AD such as the ApoE ε4 allele are highly likely to develop AD and by comparing these carriers during the asymptomatic stage to healthy non-carriers, it might provide a unique opportunity to see if subtle semantic deficits appear before the clinical onset of the dementia syndrome and whether there is any parameter reliably altered in ε4 carriers that could be used as endophenotypes in the MCI population.

Several studies have investigated the effects of the ApoE ε4 genotype on brain structure and function before and after the clinical onset of AD [e.g. (Bondi, et al., 2005; Dickerson, et al., 2005;Fleisher, et al., 2005a; Reiman, et al., 2005; Reiman, et al., 2004; Scarmeas, et al., 2004a; Scarmeas, et al., 2005a; Drzezga, Lautenschlager, Siebner, Riemenschneider, Willoch, et al., 2003; Scarmeas, et al., 2003; Bondi, et al., 2005; S. Y. Bookheimer, et al., 2000; Burggren, Small, Sabb, & Bookheimer, 2002; Corder, et al., 1997; de Leon, Convit, Wolf, Tarshish, DeSanti, et al., 2001; den Heijer, Oudkerk, Launer, van Duijn, Hofman, et al., 2002; Dickerson, et al., 2005;

Drzezga, et al., 2003; A. Fleisher, Grundman, Jack, Petersen, Taylor, et al., 2005; C. R. Jack, Jr., et al., 1998b; Lehtovirta, et al., 1995; Petrella, Lustig, Bucher, Jha, & Doraiswamy, 2002; Reiman, et al., 1996; Reiman, et al., 2005; Reiman, et al., 2004; Scarmeas, et al., 2004b; Scarmeas, et al., 2004a; Scarmeas, et al., 2005a; Scarmeas, et al., 2003; H. Soininen, et al., 1995). Young carriers of the ApoE ε4 mutation showed decreased cerebral metabolism in the areas characteristically affected in older patients with AD (Reiman, et al., 2004). In addition, despite identical performance to non-carriers, asymptomatic ApoE ε4 carriers showed decreased fMRI activation in bilateral and posterior inferotemporal regions, and increased parietal activation during naming and fluency tasks, (Smith, et al., 2002; Smith, et al., 1999) . Carriers of this genetic mutation had resting metabolism and brain blood flow abnormalities which were detectable several decades before onset of the dementia syndrome. MRI based neuroanatomical studies have yielded less clear cut findings. A number of studies have reported volumetric differences in the hippocampus, which did not reach significance levels between cognitive intact ApoE ε4 carriers and non-carriers [e.g. (Jack, Jr., et al., 1998b; Reiman, et al., 1998b)], while one study found significant reductions in hippocampal volume in carriers (Plassman, et al., 1997). Significant differences in cortical thickness of hippocampal subregions (in entorhinal cortex and subiculum, but not in the main hippocampus body and in perirhinal cortex) were found in cognitively normal carriers (Burggren, et al., 2008). Similarly, selective regional effects of the ApoE ε4 genotype on the subfield CA3 and the dentate gyrus of the hippocampus in normal ageing and AD were reported by another study (Mueller, et al., 2008).

In this regard the aim of the studies reported in these chapters is to investigate the effect of genotype (ApoE ε4) on deterioration of semantic abilities in AD and

MCI patients to verify whether the presence of this mutation might help identify the semantic measures which best differentiate normal form abnormal ageing. In particular, to check if there are significant associations between poorer semantic-linguistic ability in AD and MCI and the corresponding chromosome complement.

As a proof of concept, two small studies were carried out to test whether there was any useful association between genotypic profile and abnormal lexical-semantic performance. Finally, a larger sample study was carried out to better understand the role of this association seen in the first two studies.

4.2 STUDY I: Alzheimer's disease

4.2.1 Method

4.2.1.1 *Participants*

Twenty-nine patients with probable Alzheimer's disease of mild severity (20 males and 9 females) and twenty-five age, sex and education matched controls participated in this study. The patients underwent neuropsychiatric assessment, neurological examination and extensive neuropsychological screening. All selected patients met the NINCDS-ADRDA criteria for a diagnosis of probable AD of mild severity, (Mckhann, et al., 1984) and none had radiological evidence of ischemic brain disease. Other causes of dementia were excluded according to published clinical criteria (Brun, Englund, Gustafson, Passant, Mann, et al., 1994; McKeith, Galasko, Kosaka, Perry, Dickson, et al., 1996; Roman, Tatemichi, Erkinjuntti, Cummings, Masdeu, et al., 1993). The AD patients had a mean Mini Mental State Examination (MMSE) score of 23 (SD 2.95). All patients were examined after at least six months and had their clinical diagnosis confirmed. None of the patients in this group met the criteria for mild cognitive impairment (MCI) (Petersen, Smith, Waring, Ivnik, Tangalos, et al., 1999) as they all had difficulties in activities of daily living and\or instrumental activities of daily living at time of first referral. The participants were all British and English was their first language. All the patients recruited for this study were right-handed.

4.2.1.2 *Assessments*

Each of the patients completed a comprehensive neuropsychological test battery (see Table 4.1.), including Mini Mental State Examination (MMSE), semantic and phonemic fluency, Raven's coloured matrices, logical memory test, the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog), the neuropsychiatric Inventory (NPI) with caregiver distress scale, the geriatric Depression Scale (GDS) and Activities of Daily Living (ADL) scale (completed by patient and caregiver independently). A blood sample was also collected in order to determine their ApoE status. Two genotype category subgroups were formed:

- Category 1 (patients homozygous and heterozygous for the ApoE ε4 allele, ε4ε4\ ε3ε4) (N= 19, 8 homozygous and 11 heterozygous).

- Category 2 (patients homozygous and heterozygous for the ApoE ε3 allele, ε3ε3\ ε3ε2) (N=10, 8 homozygous and 2 heterozygous).

AD carriers had a mean MMSE score of 22.72 (SD = 2.86), AD non-carriers had a mean MMSE of 23.50 (SD = 3.17). The mean age of the carriers was 77.22 years (SD = 8.48) with a mean education of 9.61 years (SD = 1.14). The AD non-carriers had a mean age of 76.60 years (SD = 6.7) with a mean education of 13.20

years (SD = 3.42). Controls had a MMSE of 29.28 (SD = .98) with a mean education of 11.88 years (SD = 3.16) and mean age of 72.12 years (SD = 8.55).

Table 4.1. *Mean (SD) scores of the ApoE ε4 carrier patients on the neuropsychological testes compared to ApoE ε4 non-carrier patients.*

Test	AD ε4 carriers	AD ε4 non carriers	Cut-off
Mini Mental State Examination	22.72 (2.86)	23.50 (3.17)	< 27.9
Pyramids and palm Tree Test	47.41 (3.84)	47.75 (2.65)	< 49.73
Confrontation Naming	16.83 (3.35)	17.75 (1.98)	< 19.53
Verbal Paired Associates	7.66 (2.78)	7.22 (3.4)	< 8.94
Rey Complex Figure Direct Copy	17.79 (9.01)	19.16 (15.13)	< 20.45
Rey Complex Figure Delayed Copy	1.04 (1.01)	1.33 (2,33)	< 7.11
Semantic Fluency	27.00 (8.21)	26.90 (11.89)	< 42.63
Phonemic Fluency	24.31 (10.47)	26.40 (11.84)	< 29.67
Digit Span Forward	6.61 (1.33)	6.22 (0.97)	< 6.29
Digit Span Backward	4.11 (1.02)	4.22 (0.97)	< 3.94
Raven's Coloured Progressive Matrices (PM47)	21.5 (6.20)	23 (6.74)	< 28.88
Prose Memory Immediate Recall	4.5 (3.57)	4.37 (1.92)	< 12.33
Prose Memory Delayed Recall	2.5 (3.79)	3.75 (2.81)	< 12.80
Digit Cancellation	41.15 (9.56)	45.11 (13.17)	< 48.55
Stroop Test (time interference effect)	31.43 (23.96)	18.50 (13.30)	> 5.25
Token test	30.23 (2.84)	30.21 (2.84)	< 30.66
Visuocontructive Apraxia Test	13.20 (5.14)	13 (00)	< 11.38

4.2.1.3 ***Experimental procedure***

A semantic fluency task was used. Oral generation of words belonging to two categories (animals and fruits) was required. During the test administration, participants were asked to generate orally as many words as possible belonging to a given category, with the time limit of one minute per category. In order to control for order effects, the participants performed the trials in random order. Performance was scored in terms of the total number of acceptable words produced in the sixty-second trial for each category. Perseverations were not accepted.

The length, frequency, typicality, imageability and age of acquisition value for each acceptable word were determined. The data included in the analyses were the mean attributional values for the words produced by each patient.

4.2.1.4 ***Word attributes***

<u>Age of acquisition</u>

Age of acquisition (AoA) values for words were taken from the rating collected for another study in a sample of twenty elderly healthy participants (10 males, 10 females) aged between 73 and 92 years (mean = 76.2, SD = 5.53) with a mean education of 12.26 years (SD = 2.88) (Forbes-McKay, et al., 2005). Participants were asked to estimate the age (in years) at which they had learned each word. They rated the age of acquisition of 200 items (148 animals, 52 fruits) which was the total number of items generated in the course of testing by the majority of the participants in that study (4 patients and 24 controls). These controls were from a similar geographical and socio-cultural background as the patients and controls enrolled in this study. Ratings acquired in this way have been shown to correlate highly with objective measures of AoA and therefore have good validity (Morrison, Chappell, & Ellis, 1997).

Typicality

Numerous studies have shown that access to semantic knowledge (e.g. picture identification and naming) is influenced by the typicality of category exemplars [(e.g. (Holmes, Fitch, et al., 2006)]. Typical exemplars which share similar features to one another (e.g. fox and lion) and the category prototype (animal) are named faster than atypical examples (e.g. Kangaroo or snake). Raters were given a list of all items split into two categories (animal and fruit). They were requested to rate the typicality of each item by using a 7-point Likert type rating scale, from 7 (most typical) to 1 (least typical). Based on the instructions given by Larochelle, Richard and Souliers (2000), they were asked to rate how well each exemplar (e.g. apple) represented its specific category (e.g. fruit). To control for order effects, the exemplars were shown in random order to raters (Forbes-McKay, et al., 2005).

Word Frequency

The CELEX lexical database (Baayen, 1995) was consulted to obtain frequency values. This database gives two separate measures of frequency, that in the spoken language and that in the combined spoken and written language. Only the spoken values were used.

Length

Length was measured in terms of the number of letters in each word.

Imageability

Research suggests that word retrieval may be influenced by the ease with which participants can generate a mental image of a given object. Bird et al., (2000) found that objects with a high imageabilty rating were named faster by semantic dementia patients than those with lower imageability rating. Imageabilty rating (1 with great difficulty to 7 very easily) were obtained from the Oxford Psycholinguistic Database (Quinlan, 1992).

4.2.2 Results

The patients (carriers and non-carriers) did not differ in age ($F_{(2, 53)} = 2.35$, *ns*) when compared to controls, but showed a significant higher education, ($F_{(2, 53)} = 6.5$, $p < .05$)], and, lower MMSE scores ($F_{(2, 53)} = 51.82$, $p < .001$) than controls.

The data from the semantic fluency task were analysed in detail using an ANCOVA with education as a covariate followed by post hoc comparison using the Sheffe test to compare group means. The analysis compared the control group and the two patient groups on the number of words produced and on their lexical characteristics. The words generated by the two groups (patients and controls) did not differ in length ($F_{(2, 53)} = 1.68$, *ns*) or spoken frequency as recorded in the CELEX lexical database of spoken language ($F_{(2, 53)} = .193$, *ns*). Patients produced fewer words than healthy elderly and the difference was significant ($F_{(2, 53)} = 51.94$, $p < 0.01$). Moreover, patients (carriers and non-carriers) produced significantly less imageable ($F_{(2, 53)} = 4.09$, $p < 0.50$), more typical ($F_{(2, 53)} = 8.42$, $p < .00$) and earlier acquired words of their category ($F_{(2, 53)} = 21.01$, $p < .00$) when compared to controls.

Table 4.2. *Mean (SD) number of words and mean lexical characteristic values of all words produced by AD carriers, non-carriers and controls.*

	Carriers	Non Carriers	Controls	p
Number of word	16.36 (5.1)	14.70 (6.4)	35.96 (8.8)	.00
Typicality	5.64 (0.3)	5.55 (0.4)	5.17 (0.4)	.001
AoA	4.91 (0.8)	4.85 (0.9)	6.32 (0.7)	.00
Length	5.41 (0.7)	5.47 (0.3)	5.69 (5.6)	ns
Image	616.48 (9.7)	616.84 (7.5)	613.48 (5.5)	.022
Celex Spoken Frequency	8.91 (3.3)	8.94 (3.6)	8.42 (2.3)	ns

Post-hoc analyses showed that both carrier and non-carrier patients produced words which were more typical of their category, acquired earlier and more imageable than those produced by controls. However a more detailed look at the results showed that, although not significantly different from each other, the difference between the mean typicality value of AD carriers and controls was of greater significance than that between AD non-carriers and controls ($p < .001$ for carriers compared to controls; $p < .05$ for non-carriers compared to controls) (see red line in Tables 4.3. and 4.4. and Figure 4.1).

Table 4.3. *Mean (SD) number of words and mean characteristics lexical values for all words produced by AD ε4 carriers and controls.*

	ε4 carrier	Controls	P
Number of words	16.36 (5.06)	35.96 (8.79)	.00
Typicality	5.64 (.31)	5.17 (.43)	.00
Age of Acquisition	4.91 (.85)	6.32 (.73)	.00
Length	5.41 (.67)	5.69 (.48)	.22
Imageability	616.48 (9.68)	609.85 (8.23)	.02
Celex Spoken Frequency	8.19 (3.28)	8.42 (2.29)	.86

Table 4.4. *Mean (SD) number of words and mean lexical characteristic values all words produced by AD ε4 non-carriers and controls.*

	ε4 non carrier	Controls	P
Number of words	14.7 (6.41)	35.96 (8.79)	.00
Typicality	5.55 (.43)	5.17 (.43)	.034
Age of Acquisition	4.85 (.90)	6.32 (.73)	.00
Length	5.47 (.30)	5.69 (.48)	.54
Imageability	616.84 (7.5)	609.85 (8.23)	.03
Celex Spoken Frequency	8.94 (3.65)	8.42 (2.29)	.89

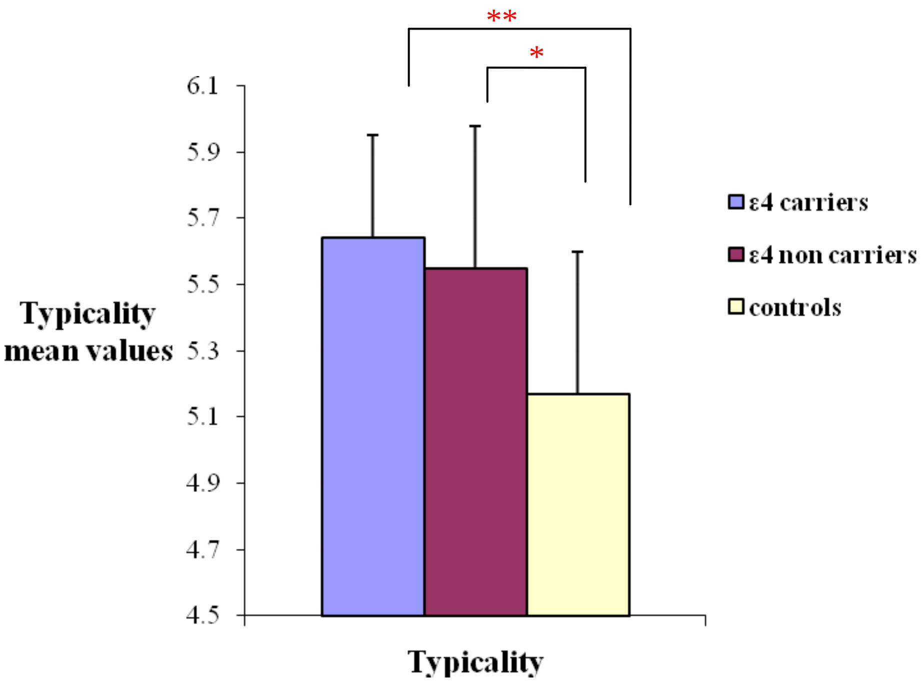


Figure 4.1. *Mean typicality values in the AD (ε4 Carriers/non-Carriers) and controls. (* * p < .001; * p< .05)*

4.2.3 Conclusion

Semantic memory impairment in AD is well documented (Chertkow & Bub, 1990; Hodges & Patterson, 1995; Hodges, Patterson, Graham, & Dawson, 1996; Hodges, Salmon, & Butters, 1991, 1992; Lambon Ralph, Patterson, & Hodges, 1997), yet much of the research has tended to focus on the underlying cause of the deficit rather than trying to discover how early in the disease process are semantic skills affected.

A study of patients with probable early AD, scoring above 23 on the MMSE (Folstein, Folstein, & Mchugh, 1975), revealed a subgroup with impairment on a range of semantic memory tasks, as well as a subgroup that performed flawlessly on these tasks (Hodges & Patterson, 1995). In a more recent study, using the same

MMSE score cut-off, Perry et al., (Perry & Hodges, 2000) reported impairments in the category fluency task from the Cambridge Semantic Battery and the Picture condition of the Pyramid and Palm Trees Test (Howard & Patterson, 1992). The development of cholinergic therapies for AD has highlighted the importance of early diagnosis and fuelled interest in the so-called MCI state, which is regarded as prodromal phase of AD (Grundman, Petersen, Ferris, Thomas, Aisen, Bennett, Foster, Jack, Galasko, Doody, Kaye, Sano, Mohs, Gauthier, Kim, Jin, Schultz, Schafer, Mulnard, van Dyck, Mintzer, Zamrini, Cahn-Weiner, Thal, et al., 2004; Petersen, Stevens, Ganguli, Tangalos, Cummings, et al., 2001).

In attempting to detect AD pathology at the very early stage, episodic memory and attentional deficits have been the focus of most research to date, with few studies including any assessment of semantic memory. However, these studies have not found any deficit specific to AD mostly because these deficits (episodic memory for example), even though sensitivity to the effects of the disease can also be found in healthy people who may not develop AD (Celsis, 2000) or may be present in individuals with mild cognitive impairment who still maintain other cognitive function and have a normal functioning in daily life (Petersen, et al., 1999). In a study by Chen et al. (2001) subjects with deficits in the domain of episodic memory, but not fulfilling clinical criteria for AD, were found to be impaired on a category fluency task. These results concur with previous studies, which found category fluency to be a very sensitive assessment of semantic impairment (Hodges & Patterson, 1995; Hodges, et al., 1996; Perry & Hodges, 2000). In contrast, Albert et al., (2001) reported normal performance on naming and category fluency task in a group of patients categorised at the .5 stage in the clinical dementia rating (CDR) scale (Hughes, Berg, Danziger, Coben, & Martin, 1982). This evidence highlights the need to use well-defined criteria for MCI and the possibility to discover sensitive indices through the semantic assessment.

Several studies have underlined the usefulness of word lexical attributes such as frequency, typicality, age of acquisition, and imageability in the detection of semantic impairment in patients with Alzheimer's disease (Forbes-McKay, et al., 2005). It has been seen that age of acquisition and frequency, for example, affect differentially the way in which healthy participants and patients answer during semantic task, with early acquired and high frequency words named more accurately by healthy elderly people (Hodgson & Ellis, 1998)and with later acquired words being more vulnerable to cognitive dysfunction (Cuetos, Aguado, Izura, & Ellis, 2002)It could be, therefore, that these lexical parameters might detects changes due to AD at a very early stage of the pathology. Some studies showed that these lexical indices effects reliably differentiated normal and pathologically ageing groups, including those affected at a minimal level, with a high degree of accuracy (Holmes, Fitch, et al., 2006; Forbes-McKay, et al., 2005; Holmes, Jane Fitch, & Ellis, 2006). Other evidence is increasingly becoming available that such subtle semantic deficits may be detectable very early in the course of AD and also in at-risk groups before sindromal diagnostic criteria are met. For example, recent studies have detected lexical semantic retrieval deficits in patients with mild cognitive impairment (Vandenbulcke, Peeters, Dupont, Van Hecke, & Vandenberghe, 2007; Adlam, et al., 2006; Duong, et al., 2006; Ostberg, Fernaeus, Hellstrom, Bogdanovic, & Wahlund, 2005), and in patients with heritable traits for early cognitive decline (Arango-Lasprilla, Cuetos, Valencia, Uribe, & Lopera, 2007;Miller, Rogers, Siddarth, & Small, 2005; Tirado, Munoz, Aguirre, Pineda, & Lopera, 2004).

In this regard this study aimed to verify the effectiveness of the lexical attribute of the words analyses as good predictors of AD in high risk patients (ε4 carriers) compared to non-carriers. This is the first study to have investigated the influence of the ApoE genotype mutations on the subtle lexical effects which characterises semantic deficits early in the course of AD. The behavioural indices from this study confirmed evidence from other studies which have shown that a qualitative deterioration of vocabulary characterises linguistic production in early AD.

The finding shows that of the four lexical semantic indices taken into account in this study, typicality appeared to be the one more severely influenced by genotype. The effect of genotype, therefore, although strong overall and clearly impacting on global cognitive decline was only partially detectable on the semantic indices. A possible explanation might be that the effect of genotype was diluted by the advanced cognitive deterioration and could only be seen as a tendency to be of a stronger magnitude in ε4 carriers.

To see whether this subtle influence might be more easily detectable at an earlier stage, the second study focused on a similar investigation in a group of mild cognitive impairment (MCI) subjects (Petersen, et al., 1999) .

4.3 STUDY II: Mild Cognitive Impairment

4.3.1 Method

4.3.1.1 *Participants*

Eighteen subjects with amnestic MCI were recruited from a referral neuropsychology centre of the Neurology Clinic at the University of Bologna. There were 11 males and 7 females in the group. Eighteen age, sex and education matched controls were also tested. All MCI subjects had a neurological examination and extensive neuropsychological screening. They met the criteria for MCI of amnestic type (Petersen, et al., 1999) as they all performed above the cut off score for any neuropsychological tests except memory tests and didn't have difficulties in activities of daily living and/or instrumental activities of daily living at time of referral. The MCI subjects had a mean Mini Mental State Examination (MMSE) score of 28.07 (SD = 2.05). The mean age of MCI subjects was of 69 years (SD = 5.64) with a mean education of 8.85 years (SD = 4.89).

The control group had a mean Mini Mental State Examination (MMSE) score of 28.71 (SD = 1.13). The mean age of the controls was 66.42 years (SD = 4.78) with a mean education of 11.57 years (SD = 4.75).

4.3.1.2 ***Assessment***

Each of the MCI participants completed a comprehensive neuropsychological test battery (see table 5.5. below). A blood sample was also collected to determine their ApoE status. Two genotype category subgroups were formed:

- Category 1 (subjects heterozygous for the ApoE ε4 allele, ε3ε4 as no patients homozygous for the ApoE ε4 were found in this group) (N = 7).
- Category 2 (subjects homozygous and heterozygous for the ApoE ε3 allele, ε3ε3/ ε3ε2) (N=11, 9 homozygous and 2 heterozygous for the ε3 allele).

Table 4.5. *Mean (SD) scores of ε4 carrier MCI on the neuropsychological tests compared to ε4 non-carrier MCI.*

Test	MCI ε4 carriers	MCI ε4 non carriers	Cut-off
Mini Mental State Examination	27.71 (2.75)	27.45 (2.38)	> 23.00
Confrontation Naming	19.16 (0.75)	17.54 (2.8)	#
Verbal Paired Associates	7.75 (2.01)	8.50 (2.71)	6
Rey Complex Figure Direct Copy	32.00 (3.28)	29.72 (7.88)	> 28.87
Rey Complex Figure Delayed Copy	13.16 (5.69)	11.27 (5.95)	> 9.46
Semantic Fluency	29.71 (15)	27.90 (8.15)	> 24.00
Phonemic Fluency	19.85 (11.52)	23.00 (8.34)	> 16.00
Digit Span Forward	6.00 (0.63)	4.72 (0.64)	3.5
Digit Span Backward	3.80 (1.09)	3.09 (0.83)	#
Raven's Coloured Progressive Matrices (PM47)	27.66 (4.03)	26.18 (6.24)	> 17.50
Visual-spatial Supra-span Learning	19.22 (6.11)	17.43 (8.45)	> 5.75
Visual-spatial Span	3.66 (0.51)	4.00 (0.44)	3.5
Digit Cancellation	48.00 (6.78)	45.09 (11.30)	> 30.00
Stroop Test (error interference effect)	0.91 (1.11)	3.45 (4.26)	< 4.24
Stroop Test (time interference effect)	22.75 (10.81)	34.27 (15.67)	< 36.92
Token test	33.66 (1.47)	32.04 (4.18)	26.25

data not available

4.3.1.3 ***Experimental Procedure***

Materials, measures and procedures as in study I

N.B. One additional lexical measure was used i.e. familiarity.

4.3.1.4 ***Word attributes***

As in study I with the exception of the additional attribute of familiarity.

<u>Familiarity</u>

Raters were given a list of items split into two categories (animals and fruit). They were given a 7-point rating scale, from 7 (very familiar) to (little familiar). They were asked to rate how familiar they were with a particular item. To control for order effects, the exemplars were shown in random order to raters.

4.3.2 Results

The MCI (carriers and non-carriers) and control groups did not differ in age ($F_{(2,27)}$ = -1.14, *ns*) education ($F_{(2,27)}$ = 2.93, *ns*) and MMSE scores ($F_{(2,27)}$ =0.83, *ns*). The data from the semantic fluency task were analysed in detail using a one way ANOVA followed by post hoc comparisons using the Sheffe test to compare group means. The analysis compared the control group and the two MCI subgroups on the number of words produced and on their lexical characteristics. Looking at the mean lexical characteristics of words produced by the MCI ε4 carriers it seems that this group tended to generate more typical, familiar and earlier acquired words of their category than those of non-carriers and controls (see Figures 4.2 and 4.3.). The differences did not reach statistical significance apart for number of words, where ε4 non-carrier produced significantly more words than controls. However, where supporting the reason for each comparison (that is ε4 carriers vs control and ε4 non-carriers vs controls for each characteristics) it appeared that the difference between the means of the non-carriers and the controls was smaller than that of carriers and the controls (see Tables 4.6 and 4.7). Overall, therefore, it seems that ε4 carriers produced words which are more typical, familiar and acquired earlier than controls which in turn mirrored the same lexical pattern when compared to ε4 non-carriers (typicality and familiarity: ε4 carriers > controls > ε4 non-carriers; Age of Acquisition: ε4 carriers < controls < ε4 non-carriers).

Table 4.6. *Mean (SD) number of words and mean lexical characteristic values of all words produced by the MCI ε4 carriers and control.*

	ε4 carrier	Controls
Number of words	29.71 (15.00)	21.85 (5.46)
Typicality	4.53 (1.10)	4.20 (0.25)
Age of Acquisition	4.50 (1.57)	4.77 (0.48)
Length	6.33 (0.36)	6.10 (0.33)
Familiarity	4.57 (1.10)	4.30 (0.18)

Table 4.7. *Mean (SD) number of words and mean lexical characteristic values of all words produced by the MCI ε4 non-carriers and control.*

	ε4 non carrier	Controls
Number of words	33.33 (5.97)	21.85 (5.46)
Typicality	4.07 (0.21)	4.20 (0.25)
Age of Acquisition	5.23 (0.58)	4.77 (0.48)
Length	6.14 (0.43)	6.10 (0.33)
Familiarity	4.04 (0.26)	4.30 (0.18)

Figure 4.2. *Mean values in MCI. No significant differences were found. A strong trend in the expected direction was present for Typicality.*

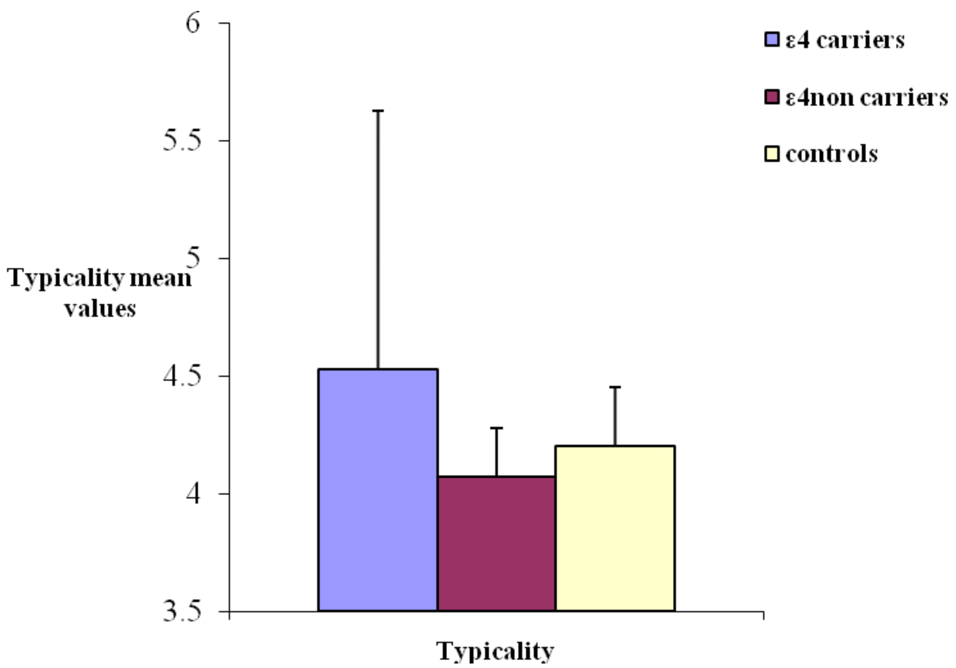
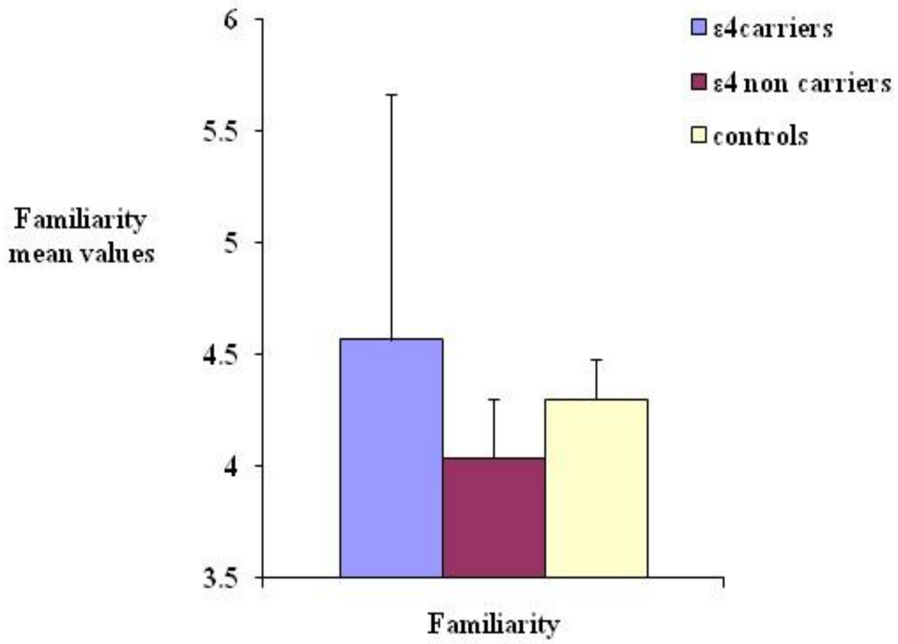
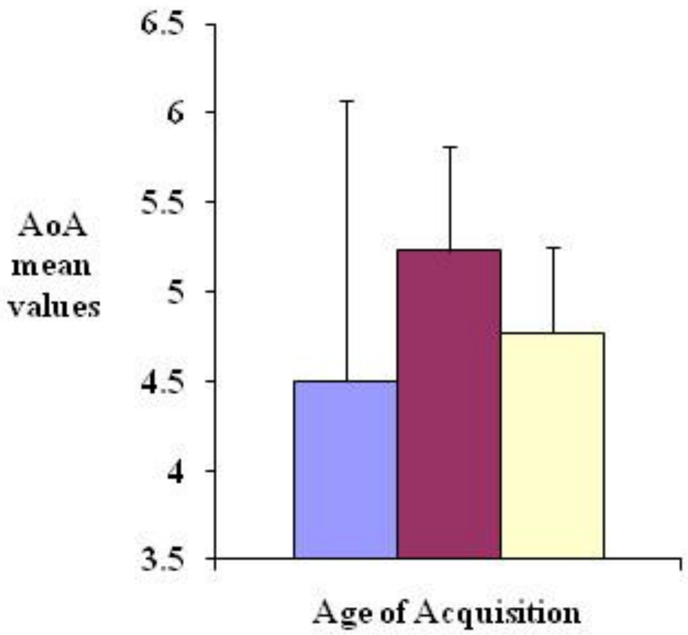


Figure 4.3. *Mean values in MCI. No significant differences were found. A strong trend in the expected direction was present for Familiarity (a) and Age of Acquisition (b).*

a)



b)



4.3.3 Conclusion

The findings showed an association between lexical attributes of the words and the presence of at least one allele of the Apolipoprotein ε4 although group differences were not significant. However, it appears to be the rule, rather than the exception, that those patients who present with an isolated memory problem in the clinic reveal more widespread cognitive dysfunction when tested with more sensitive experimental measures.

Many researchers [e.g. (Petersen, et al., 1999; Tapiola, et al., 2008)] consider the group with a diagnosis of MCI to be in a transitional stage along a continuum between normal aging and dementia on the basis of memory performance. Duong and colleagues (2006) have reinforced and expanded this notion by describing a continuum for lexical-semantic measures in a group of patients clinically diagnosed as “amnestic” MCI (Petersen, et al., 1999). This study corroborates recent epidemiological observations that subtle cognitive impairments, such as language, may co-occur with the readily observed memory impairments (Adlam, et al., 2006; Duong, et al., 2006; Petersen, et al., 1999; Ritchie, Artero, & Touchon, 2001). Of interest is also the recent evidence from cognitive performance on lexical/semantic tasks in healthy individuals who carry genetic mutations for familial AD. Poorer semantic performance, when compared to non-carriers, was reported in otherwise asymptomatic carriers of a genetic mutation for familial AD (E280A presenilin-1 gene) (Arango-Lasprilla, et al., 2007).This suggests, for the first time, a possible link between language skills and the ApoE genotype mutations to account for these early lexical-semantic impairments. The results are encouraging but larger samples of both MCI subjects and controls are needed. If confirmed in larger groups, semantic indices associated with genetic mutations may offer a useful prognostic indicator in mild cognitive impairment. Moreover, these very preliminaryfindings go in a similar direction as larger studies of other cognitive abilities in healthy elderly (Lessov-Schlaggar, Swan, Reed, Wolf, & Carmelli, 2007; Tupler, et al., 2007).

4.4 STUDY III: Influence of ApoE status on lexical-semantic skills in Mild Cognitive Impairment

4.4.1 Introduction

As already discussed above and in the previous chapters, Alzheimer's disease is a very complex pathology whose aetiology is still the object of research in many studies. Different approaches have been used in order to find a clearer pathway that lead to the underlying mechanism of the pathology. The analyses of cognitive functions supported by neuroimaging techniques has shown which neuronal substrates and cognitive characteristics are mostly associated with this disorder at the very early stage (for a review see Chapter 1). Moreover, genetic studies have identified the ApoE ε4 allele as the major risk factor for sporadic late-onset AD and have investigated the effects of this genotype on brain structure and function before and after the clinical onset of AD (Bookheimer, et al., 2000; Jack, Jr., et al., 1998). The purpose of these studies has been that of finding some specific features that could improve accuracy in AD diagnosis. This evidence encouraged the search for preclinical markers in carriers of this mutation, focused mainly on those cognitive functions affected in people at the early stage of the disease. Among the possible endophenotypes, episodic memory and language deterioration have been the major candidates. However, studies of memory changes in AD and in predementia individuals have not brought to light any clear preclinical indicators, probably due to the fact there are no unique features of the AD pathological changes. In contrast, evidence of lexical-semantic deterioration in AD at the preclinical stage and in people at risk have suggested the utility to further investigate the role of these indices in predicting abnormal decline. In particular, studies using semantic fluency tasks as possible AD discriminatory clinical tools found interesting results. Qualitative analyses of category fluency performance highlighted the role of the lexical characteristics of the words in the detection of semantic impairment in AD patients (Forbes-McKay & Venneri, 2005), in MCI (Vandenbulcke, et al., 2007; Adlam, et al., 2006) and in patients with a genetic trait for familial AD (Cuetos, et al., 2007). This converging evidence brought us to investigate whether and to with extent subtle semantic deficits associated to a high probability of developing AD could be considered as a reliable preclinical indicator which might ensure greater diagnostic accuracy at this early stage.

The first study focused on searching for a lexical endophenotype in mild to moderate AD, and observed a qualitative deterioration of vocabulary in AD but by this stage the effect of genotype was only detectable on the semantic indices as a tendency to be greater impact in ε4 carriers (see section 4.2.5.). The impact of the ε4 mutation is age dependent, and shows its highest peak by the age of 70 to dissipate on the way to 80 (Breitner, et al., 1999). The high mean age of our participants (77.22) might therefore have influenced the effect of the ApoE ε4 allele on lexical measures. Moreover, given the dose-response way in which ε4-related deficits are determined (ε4ε4> ε4ε3> ε3ε3), another possible factor masking or attenuating the effect could have been the absence of ε4 homozygoutes in our sample, which might have contributed to weakening the lexical difference between the carrier and non-carriers AD subgroups.

The aim of the second study was to ascertain whether any ApoE ε4 mutation effect on lexical semantic skills could be more clearly identified at the mild cognitive impairment stage, when the neuropathological effect of the disease should be more limited and confined. The results showed again a stronger effect of ε4 allele, although, lack of statistical power prevented the detection of a significant difference between carriers and non-carriers.

The aim of this third study, therefore, was to characterise the relative contribution of genetic influence to individual differences in both cross sectional performance and decline of linguistic abilities in a larger amnestic MCI sample. Semantic competency was assessed by determining the lexical attributes (i.e. age of acquisition, typicality, familiarity) of words produced in a category fluency task, as in the earlier studies.

4.4.2 Method

4.4.2.1 ***Participants***

Thirty subjects with amnestic MCI were recruited from a large pool of referrals to the specialist referral unit for memory and other cognitive disorders at the University of Parma, Italy. There were 14 males and 16 females in the group. Twenty two age and education matched controls (4 males and 18 females) were also tested. A diagnosis of amnestic MCI was reached based on published criteria. All MCI subjects had a full clinical assessment including neurological examination and extensive neuropsychological screening. They met the criteria for MCI of amnestic type (Petersen, et al., 2001) as they all performed above published cut-off scores on all neuropsychological tests except for tests of long term memory. None had any difficulties in activities of daily living and/or instrumental activities of daily living at time of referral. To exclude the presence of dementia, all individuals had comprehensive clinical and neuropsychological examinations (including assessment of activities of daily living), and did not meet the international published guidelines for the diagnosis of different types of dementia (McKeith, et al., 1996; Roman, et al., 1993; Brun, et al., 1994; Mckhann, et al., 1984). Individuals were included only if there was no neuroimaging evidence of cortical or subcortical vascular lesions on CT or MRI scan and if there was no history of hypertension, diabetes mellitus, transient ischemic attacks, or cardiovascular problems. Additional exclusion criteria included the presence of significant symptoms of depression, a history of psychiatric disorders and treatment with antipsychotic or psychoactive medication at the time of investigation. A blood sample was also collected to determine the APOE status of both MCI and control participants. On the basis of their genetic profile the MCI sample was divided in a ε4 carrier subgroup including 18 subjects (8 male and 10 female), all heterozygous for the ApoE ε4 allele (ε3ε4) and a non-carrier subgroup including 12 subjects (6 male and 6 female) homozygous and heterozygous for the ApoE ε3 allele (ε3ε3/ε3ε2). No ApoE ε4 carriers were found in the control group. MCI carriers had a mean MMSE score of 26.61 (SD = 2.22), a mean age of 70.61 years (SD = 9.61) and a mean education of 10.94 years (SD = 5.17). MCI non-carriers had a mean MMSE score of 27.58 (SD 1.56), a mean age of years 72.50 (SD = 9.11) and a mean education of 7.83 years (SD = 4.20). The control group had a mean MMSE score of 28.95 (SD = 0.84), a mean age of 66.59 years (SD = 9.23) and a mean education of 10.05 years (SD = 4.41). The same exclusion criteria used in the recruitment of the MCI sample were adopted for the healthy older adult sample. The same international guidelines used to exclude the presence of a dementia syndrome or to ascertain the presence of mild cognitive impairment in the MCI sample were also used in the recruitment of the healthy older adult sample. The study received local ethics committee approval and all MCI and control participants gave informed consent to their participation in the study.

4.4.2.2 ***Neuropsychological assessment***

All MCI subjects completed a comprehensive neuropsychological test battery. The neuropsychological test battery included the MMSE, category and letter fluency tasks; the Rey complex figure task (direct and delayed copy), the Raven standard coloured progressive matrices (PM 47), the prose memory task; the visual-spatial supra-span learning test; a digit cancellation task and the Stroop test.

4.4.2.3 ***Experimental Procedure***

A category fluency task including the categories of fruit and animals was used. Performance was evaluated by collating the total number of words produced for these two categories and by determining the lexical attributes (length, typicality, familiarity and age of acquisition) for each acceptable word. Patients and controls performed two 60 second trials (one for animals and one for fruit) during which they were requested to produce orally as many exemplars belonging to the target category as possible. Each of the items produced for this task was then scored in terms of lexical attributes (see below). The data included in the analyses were the mean attributional values of the words produced by each person.

4.4.2.4 ***Word lexical semantic attributes***

Age of acquisition

Age of acquisition (AoA) values for words were obtained by asking a sample of 46 healthy older adults [25 females, 21 males, mean age 68.87 years (SD = 7.68), mean education 9.76 years (SD = 5.09), mean MMSE 28.69 (SD = 1.03)] to rate the AoA of 289 words (66 fruit and 223 animal words) produced by all MCI and control participants in this study following the procedure reported in the study by Forbes-McKay et al., (2005). Each participant was presented with a random list of all 289 items and asked to estimate the age (in years) at which they had learned a given word and its meaning in spoken or written form. Harmonic mean AoA ratings for each item were calculated and used in the analyses. These raters were from a similar geographical and socio-cultural background as the participants enrolled in this study. Ratings acquired in this way have been shown to correlate highly with objective measures of AoA and therefore have good validity (Morrison, et al., 1997).

Typicality

See details in study I page 127.

Familiarity

Raters were given a list of items split in two categories (animals and fruit). They were given a 7-point rating scale, from 7 (very familiar) to 1 (little familiar). They were asked to rate how familiar they were with a particular item. To control for order effects, the exemplars were shown in random order to raters.

Length

Length was measured in terms of the number of letters in each word.

4.4.3 Results

There was no significant difference in age ($F_{(1, 50)} = 3.37$ *ns*) nor in education ($F_{(1, 50)} = 0.07$, *ns*) between the MCI sample and the controls. No significant differences were found between the two MCI ε4 carriers/non-carriers subgroups and the controls for age ($F_{(2, 49)} = 1.81$, *ns*) and education ($F_{(2, 49)} = 1.65$, *ns*).

4.4.3.1 *Neuropsychological assessment*

The means and standard deviations of each MCI subgroup's score on the tests included in the standard neuropsychological battery are shown in Table 4.8. (see below). Although the MMSE score in the MCI group remained well above cut-off on this screening test, there was a significant difference in mean MMSE scores between the MCI group and the controls ($F_{(1, 50)} = 18.23$, $p < .001$). A comparison between MMSE scores in MCI ε4 carriers, MCI non-carriers and controls revealed that there was a significant difference between the MCI subgroups and controls ($F_{(2, 49)} = 10.74$, $p < .001$), but only the mean MMSE score of the MCI ε4 carrier subgroup was significantly different from controls ($p < 0.001$), while that of MCI non-carriers was not. When directly compared, the scores of the two MCI subgroups (ε4 carrier/non-carrier subgroups) did not differ significantly.

The scores of MCI ε4 carriers and MCI non-carriers on each test in the neuropsychological assessment were compared with an ANOVA. There were no significant differences between the two genetically defined subgroups in any of the tests included in the battery, except for scores on the category fluency task ($F_{(1,28)} = 10.22$, $p < 0.01$). Individual scores of MCI ApoE ε4 carriers/non-carriers on the prose memory test fell below the established cut-off for the Italian population, while scores on all other tests in the neuropsychological battery were in the normal range and above the established cut-off for the Italian population.

Table 4.8. *Mean (SD) scores of MCI ApoE ε4 carrier and non-carriers on the screening neuropsychological test.*

Test	**MCI ε4 carriers**	**MC non carriers**	**Cut off score**
Mini Mental State Examination	26.61 (2.22)	27.58 (1.56)	< 23.00
Prose Memory Test	4.89 (2.02)	6.50 (2.64)	< 7.50
Rey Complex Figure - direct copy	29.64 (4.92)	29.75 (5.77)	< 28.87
Rey Complex Figure - delayed copy	10.41 (6.05)	11.79 (4.51)	< 9.46
Semantic Fluency *	24.60(10.05)	35.36 (6.20)	< 24.00
Phonemic Fluency	23.12 (9.72)	27.55 (8.39)	< 16.00
Raven's Coloured Progressive Matrices (PM47)	24.75 (4.61)	26.82 (4.60)	< 17.50
Visual-spatial Supra-span Learning	13.45 (8.07)	12.48 (9.47)	< 5.75
Digit Cancellation	43.78(11.35)	50.00 (6.47)	< 30.00
Stroop Test (error interference effect)	1.75 (1.44)	1.44 (1.50)	> 4.24
Stroop Test (time interference effect)	30.05(12.11)	34.17(19.98)	> 36.92

*Significant group difference $p < 0.01$

4.4.3.2 ***Lexical-semantic assessment***

The mean number of words and mean lexical-semantic values for the words produced by the two MCI subgroups and by the control group in the category fluency task are shown in Table 4.9.

Table 4.9. *Mean (SD) number of words and lexical characteristic values of all words produced by the MCI ApoE ε4 carriers, non- carriers and controls on the category fluency task.*

Word characteristics	ε4 carrier MCI	Non carrier MCI	Non carrier Controls
Number of words	19.72 (4.56)*	24.25 (4.37)*	32.18 (6.69)
Age of acquisition	4.97 (0.42)*#	5.77 (0.47)	6.25 (0.87)
Typicality	4.44 (0.27)	4.21 (0.23)	4.32 (0.28)
Familiarity	4.15 (0.36)*	4.04 (0.26)	3.83 (0.35)
Length	6.00 (0.42)	6.25 (0.26)	6.18 (0.31)

* Significantly different from controls
# Significantly different from non carriers

There was a significant difference between the MCI subgroups and the control group in the mean number of words produced in the fluency task ($F_{(2, 49)} = 25.83$, $p <$ 0.001). Post-hoc analysis showed that both MCI subgroups were significantly different from controls ($p < 0.001$ for both comparisons), but they did not differ from each other. Mean word length of both the MCI subgroups and the controls did not differ ($F_{(2, 49)} = 2.24$, *ns*), nor did mean word typicality ($F_{(2,49)} = 2.72$, *ns*). A significant difference between MCI ε4 carriers/non-carriers and controls was found for word familiarity ($F_{(2, 49)} = 4.55$, $p < 0.02$). Post-hoc analysis showed that the mean word familiarity of MCI ε4 carriers differed significantly from that of controls ($p < 0.02$), but there was no significant difference between MCI non-carriers and controls nor between the MCI ε4 carrier/non-carrier subgroups. Mean AoA values of the MCI subgroups and controls were significantly different ($F_{(2, 49)} = 18.56$, $p < 0.001$). Post-hoc analysis, however, showed that the mean AoA values of words produced by MCI ε4 carriers were significantly lower than those of both MCI non-carriers ($p < 0.005$) and controls ($p < 0.001$). No significant differences were found between MCI non-carriers and controls. Multiple analyses of covariance were also carried out to rule out any possible spurious influence of age, education, gender or MMSE score difference on the number of words and on the lexical parameters of the words produced in the category fluency task. Demographic variables and MMSE scores were all included as covariates in the analyses. Significant group differences remained for number of words ($F_{(2,45)} = 13.31$, $p < 0.001$), age of acquisition ($F_{(2,45)} = 9.08$, $p = 0.001$) and for familiarity ($F_{(2,45)} = 3.91$, $p < 0.05$), but no significant group difference was found for word length ($F_{(2,45)} = 0.84$, *ns*) or typicality ($F_{(2,45)} = 2.99$, *ns*), although in this latter case the p value very closely approached significance level ($p = 0.06$).

4.5 Discussion

Although a number of studies have separately examined ApoE ε4 in relation to the probability of developing AD and the influence that this gene may have on the cognitive performance in healthy subjects, no study has evaluated the predictive value of this genotypic mutation and the lexical attributes of individuals' vocabulary in combination. We found that spontaneous language in MCI ε4 carriers was very much impoverished compared to healthy controls and their verbal output was characterised by significant lexical effects. There were a significantly smaller number of words produced, and a significant difference in the lexical semantic characteristics of their residual word production. The words generated by MCI ApoE ε4 carriers were earlier acquired, more familiar and more typical of the semantic category than the words generated by healthy controls. The age of acquisition value and the number of words produced were the parameters showing the strongest effects, even when the effect of any difference in MMSE scores and/or demographic variables between the MCI ε4 carriers/non-carriers and controls were partialled out. Words produced by MCI ε4 carriers were significantly earlier acquired than those produced by controls, but also significantly earlier acquired than those produced by MCI non-carriers. For all other parameters, the performance of the two MCI subgroups was significantly worse than that of controls but there was no significant difference between the two genetically defined MCI subgroups. Earlier work (Forbes-McKay, et al., 2005) showed that the number of words produced in a category fluency task and their lexical characteristics (typicality, length, frequency and age of acquisition) significantly discriminated AD patients from controls, with the mean age of acquisition of words generated, correctly classifying 95% of controls and 88% of patients. Moreover, the small number of words generated by AD compared to controls did not significantly contribute to increasing the discriminatory power of the AoA word attribute. This was shown through a further analysis carried out by these authors to determine whether the differences in word characteristics between patients and controls remained when the mean attributional values were derived from a maximum of 10 words per participant (no more than the first five words produced in each of the two categories). It appears, therefore, that age of acquisition is a lexical parameter which is very sensitive even at a minimal level of neuropathological deficit.

MCI ε4 carriers had significantly poorer performance compared to MCI non-carriers on the category fluency test, but there was no difference between the groups on the letter fluency test. Similar results have been reported by Venneri et al., (2008) with mild clinical AD. This finding suggests that poorer performance in the semantic fluency task reflects a semantic (temporal) rather than executive (frontal) deficit. This dissociation might reflect the operation of two partly overlapping but dissociable neural systems for the two tasks. The left inferior frontal gyrus has been consistently associated with both phonologic and semantic operations in functional neuroimaging studies, but a recent review supports distinct dorsal-ventral locations for phonological and semantic processes within this structure (Costafreda, Fu, Lee, Everitt, Brammer, et al., 2006). fMRI studies have also suggested that the medial temporal lobe (especially the hippocampal formation and posterior parahippocampal gyrus) is required for the process of retrieval by category (Pihlajamaki, et al., 2000). Letter fluency performance in contrast is known to rely on left frontal cortical regions and there is evidence that it can be affected by lesions in the white matter in this area (Fernaeus, Almkvist, Bronge, Ostberg, Hellstrom, et al., 2001). Morphometric evidence from a voxel based correlational study of mild AD has established a role for structures of the anterior medial temporal cortex, the perirhinal cortex in particular, in category fluency but not in letter fluency (Venneri, et al., 2008). More severe deficits in category than letter fluency in carriers of the ApoE ε4 mutation might, in turn, mean a more severe neuropathological burden in medial temporal regions. In MCI non-carriers the presence of more modest lexical-semantic deficits suggests that, despite similar MMSE and episodic memory deficits, there might be sufficient residual neural capacity in the perirhinal and anterior temporal cortex which can still support relatively efficient retrieval from long term semantic memory. This hypothesis is supported by other studies which have highlighted ApoE-related differences in cerebral structures, brain blood flow and metabolism, and cerebral activation in the medial temporal structures (including hippocampus, cingulate areas etc.), even in young healthy carriers [e.g. (Scarmeas & Stern, 2005)]. Mediotemporal limbic structures and especially parts of the hippocampal complex are of course the areas which have been found severely atrophic in MRI studies of AD patients, with high levels of atrophy detectable years before a formal diagnosis is made (Fox & Schott, 2004). There is evidence that grey matter loss in medial temporal structures, especially perirhinal and parahippocampal cortex, as well as neocortical regions in the anterior temporal pole would result in degraded semantic outputs in patients in the early stage of AD. Such outputs are characterised by strong lexical effects (age of acquisition and typicality effect especially) Venneri, et al., 2008). It is therefore possible that MCI carriers might have more selective damage to the perirhinal cortex and other components of the memory retrieval system, and for this reason they show a more degraded semantic output with stronger lexical effects (age of acquisition especially) than non-carriers. An alternative explanation might be that the neuroanatomical substrate supporting retrieval from long term semantic memory is selectively sensitive to the earliest effects of the ApoE ε4 burden and its apparent interaction with AD pathology during the life course. This latter hypothesis finds some support in the evidence of lower metabolic activity in regions of the parietal and temporal cortex strongly associated with lexical semantic representations in asymptomatic carriers of the ApoE ε4 mutation (Reiman, et al., 2004).

Finally, the significant association between the ApoE ε4 mutation and an accentuated semantic deficit in MCI subjects might be of some clinical relevance in this at risk population. A more sophisticated analysis of cognitive performance using tests like the category fluency task may provide clinically relevant early indicators of pathological brain ageing in individuals at greater risk of AD and trigger more detailed neuropsychological investigations in those subjects with poorer performance.

CHAPTER 5 Interplay between genetic factors brain atrophy and semantic-lexical deficits

5.1 Introduction

Alzheimer's disease is a challenging pathology and the reason why it is so difficult to make a diagnosis and to plan proper cognitive and pharmacological intervention is because of the heterogeneity of its symptoms and the non-peculiarity of its preclinical signs which can be seen even in people who will never develop this pathology. Multiple genes and environmental factors are believed to be involved in the pathogenesis and development of the disease through a complex interplay that is still largely unknown. Therefore, intensification of research in this field, focusing on the preclinical markers of this pathology, has arisen. To date, a large body of evidence has already shown that cognitive deficits and neuropathological hallmarks can be detected very early in the course of AD and even before the stage of clinical onset. Among the prodromal symptoms, marked neuronal loss in the medial and anterior temporal regions, such as the presence of neurofibrillary tangles and senile plaques, can be observed very soon in the course of the disease (Gomez-Isla, Irizarry, Mariash, Cheung, Soto, et al., 2003). Volumetric MRI studies showed hippocampal atrophy before the onset of the dementia (Fox, et al., 1996a; Jack, Jr., et al., 1999; Visser, et al., 1999a) with progressive deterioration along the continuum toward the disease (Fox, et al., 1996a). Cognitive deficits can also be seen at the preclinical stage of AD together with neuropathological changes. Besides the well known memory problems which have been the focus of many research studies for decades, language represents another useful target of investigation in order to better understand the cognitive differences between healthy people who will never develop AD and those at risk of developing the disease. As mentioned in the previous chapters, language impairment occurs early in AD, affecting grammatical structure and vocabulary, (Forbes-McKay, et al., 2005), verbal ability (Convit, de Asis, de Leon, Tarshish, De Santi, et al., 2000; Jacobs, et al., 1995a; B. J. Small, et al., 1997b) and verbal fluency (Alberca, et al., 1999). Detailed linguistic analysis shows an abnormal pattern in semantic retrieval that characterises the residual linguistic production of these patients. Forbes-McKay et al., (2005) showed how the lexical characteristics of words such as age of acquisition (AoA), imageability and typicality might be affected in the residual linguistic skills observed in patients in the early stage of the disease. Venneri et al., (, 2008) found that these lexical semantic deficits correlate strongly with medial temporal atrophy and discriminate normal from abnormal cognitive decline. These language deficits which affect AD very early in the course of the pathology seem to be detectable already at the Mild Cognitive Impairment stage. Most studies, for example, using a fluency task as a measure of individuals' residual lexical abilities, showed the diagnostic utility of this instrument in differentiating MCI and healthy people who will develop AD from those who won't (Risacher, et al., 2009; Auriacombe, Lechevallier, Amieva, Harston, Raoux, et al., 2006; Blackwell, Sahakian, Vesey, Semple, Robbins, et al., 2004; G. W. Small, La Rue, Komo, Kaplan, & Mandelkern, 1997; Vogel, et al., 2005). Moreover evidence from VBM studies, which depicted a pattern of atrophy in brain areas typically affected by AD neuropathological changes in amnestic MCI (aMCI) who progress to AD, showed the importance of using this technique as a necessary tool toward a more vigorous approach to the detection of this disease (Baron, Chetelat, Desgranges, Perchey, Landeau, et al., 2001; Bell-McGinty, et al., 2005; Bozzali, et al., 2006a; Davatzikos, Fan, Wu, Shen, & Resnick, 2008; Frisoni, Testa, Zorzan, Sabattoli, Beltramello, et al., 2002; J. Jack & Myette, 1997; Karas, Scheltens, Rombouts, Visser, van Schijndel, et al., 2004; Risacher, et al., 2009; Whitwell, et al., 2008). Unfortunately at this preclinical level a specific cognitive assessment linked to neuroimaging results, even though increasing the accuracy of diagnosis of AD, is still not enough to make its diagnostic value certain mainly due to the small proportion of amnestic MCI who progress to AD. A relatively new approach to investigate complex neurological disorders consists of linking specific genotypic factors to the behavioural and neuropathological expressions of that pathology. Only few studies have investigated the relationship between genetic susceptibility to AD (genetic risk factors) and the potential presence of the disease (potential markers) (Schoonenboom, Visser, Mulder, Lindeboom, Van Elk, et al., 2005). If mediotemporal atrophy already detected in the MRI and linguistic semantic deficits can be considered as valid biomarkers for AD, the presence of them in people at risk for AD could increase accuracy of diagnosis by showing predictable patterns.

To date, the ApoE ε4 is one of the major known risk factors for late-onset AD. It is present in higher frequency in AD subjects than it is in the normal population (Saunders, et al., 2000), lowers the onset of the disease in a dose-dependent way(Goldstein, Ashley, Gearing, Hanfelt, Penix, et al., 2001) and several studies have shown the importance of this mutation in preclinical diagnosis (Reiman, et al., 2001; Reiman, et al., 1996; Reiman, et al., 2005; Reiman, et al., 2004; Scarmeas, et al., 2002; Scarmeas, et al., 2004a; Scarmeas, et al., 2005a; Scarmeas, et al., 2003; Scarmeas & Stern, 2006). PET studies during rest have shown abnormal cerebral metabolism for glucose in healthy young carriers in the same areas usually affected by AD neuropathology (Reiman, et al., 2004). fMRI studies showed altered patterns of brain activation in the absence of any deficits in performance during a semantic memory task in healthy high risk people carrying the ApoE ε4 mutation (Bookheimer, et al., 2000; Seidenberg, Guidotti, Nielson, Woodard, Durgerian, et al., 2009; Smith, et al., 1999, 2002; Trivedi, et al., 2006; Woodard, Seidenberg, Nielson, Antuono, Guidotti, et al., 2009; Bondi, et al., 1995) . Interestingly the presence of this allele seems to influence also cognitive performance of healthy subjects since childhood (Jacob Raber, 2009). Findings of structural MRI studies are mixed. Some showed no significant difference in atrophy patterns between healthy carriers and non-carriers (Reiman, et al., 1998b), and only one showed a reduced hippocampal volume (Plassman, et al., 1997). Burggren et al. (2008) found reduced cortical thickness in hippocampal sub-regions in healthy ApoE ε4 carriers, whilst Muller et al., (Mueller, et al., 2008)found a regionally selective effect on CA3 and dental gyrus in normal aging and AD. Finally, Filippini et al.(2009a) showed grey matter volume (GMV) reduction in MLT structures, including the amygdala, hippocampus, parahippocampal gyrus and temporal fusiform cortex. ApoE ε4 is also involved in the cognitive decline seen in healthy subjects and Mild Cognitive Impairment. Caselli et al., (2009) showed that middle aged healthy subjects homozygotes for ApoE ε4 have higher rates of cognitive decline than ApoE ε4 heterozygotes or non-carriers before the diagnosis of MCI or AD. As in previous research with established clinical AD (Venneri, et al., 2008; Forbes-McKay, et al., 2005; Venneri, et al., 2005) in our behavioural study (see Chapter 4 section 4.4) it was found that MCI ε4 carriers had poorer semantic performance than controls, while this was not the case for non-carriers. Another example of subtle semantic deficits in healthy carriers comes from the study of people carrying a genetic mutation for familiar AD (Arango-Lasprilla, Rogers, Lengenfelder, Deluca, Moreno, et al., 2006). Poor semantic performance, when compared to non-carriers, was found in asymptomatic carriers. These findings seem to provide in vivo evidence of a role of this mutation in AD; less clear, though, is its role in modulating the expression of the disease. Data in partial disagreement come from post–mortem and in vivo studies with the former reporting greater accumulation of AD pathological hallmarks in the neocortex of patients ε4 carriers than those without this allele (Tiraboschi, et al., 2004) and the latter reporting greater atrophy in the hippocampus, enthorinal cortex and temporal lobe with relatively preserved frontal volume in carriers, suggesting a region specific effect of the ε4 allele on brain atrophy rather than an overall greater disease severity (Hashimoto, et al., 2001; Pievani, et al., 2009; Geroldi, et al., 1999) .

On the basis of these results it seems reasonable to hypothesise that the presence of the "semantic endophenotype" in people carrying the ApoE vulnerability mutation might be associated with early atrophy in areas affected by neuropathology due to AD and involved in semantic memory retrieval (MLT regions including areas surrounding the hippocampus). In this section two studies are presented with the first aiming to test whether poorer lexical semantic competency in MCI ε4 carriers is related to more extensive grey matter volume differences in regions supporting semantic retrieval from long term memory, by comparing grey matter volume in MCI ε4 carriers/non-carriers and in non-carrier controls. In addition, a second study was carried out to better clarify the ApoE related patterns of atrophy over the whole cortex by direct volumetric comparisons in mild AD ε4 carrier/non-carrier patients and in MCI ε4 carrier/non-carrier subjects.

5.2 STUDY I: The relationship between ApoE ε4 genotype, brain volume and poorer semantic skills in patients with MCI.

5.2.1 Aim of the study

The previous study (see Chapter 4 section 4.4) showed that a significant proportion of phenotype variability in performance on the fluency task was influenced by genetic factors. Individuals with MCI carrying the ApoE ε4 mutation seem to produce early acquired words than MCI non-carriers when compared to controls. Moreover, these semantic lexical deficits are one of the earliest characteristics of residual language in patients with AD (K. E. Forbes-McKay, et al., 2005) and it seems to correlate with atrophy of the neuronal substrates which are affected by neuropathological changes (limbic area) very early in the course of the disease (A. Venneri, et al., 2008). In addition, VBM studies have been very informative and have detected patterns of atrophy in aMCI who progress to AD in brain areas typically affected by AD neuropathological changes (Risacher, et al., 2009; Whitwell, et al., 2008).

Supported by significant results of the previous studies showing that the sensitivity of lexical semantic parameters in detecting sublte deficits is more prominent in aMCI carriers and therefore that those parameters might be used as possible endophenotype of AD, the investigation was extened to the anatomical level. This approach was further supported by neuroimaging evidence showing the impact of the ApoE ε4 burden in healthy young people on areas usually affected by AD neuropathology (Woodard, et al., 2009; Reiman, et al., 2004; C. D. Smith, et al., 2002). The aim of this study, therefore, was to investigate whether a differential pattern of grey matter volume loss was detectable in aMCI ε4 carriers/non-carrier and to identify the anatomical correlation of the age of acquisition (AoA) effect in these genetically determined subgroups of aMCI patients.

5.2.2 Methods

5.2.2.1 *Participants*

Eighteen subjects with aMCI were recruited from a referral neuropsychology centre of the Neurology Clinic at the University of Modena and Reggio Emilia, Italy. There were 11 males and 7 females in the group. Twelve education matched controls were also tested. All MCI subjects had a full clinical assessment including neurological examination and extensive neuropsychological screening. They met the criteria for aMCI(Petersen, et al., 2001) as they all performed within the cut off for all neuropsychological tests except memory tests and didn't have difficulties in activities of daily living and/or instrumental activities of daily living at time of referral. Additional exclusion criteria were the presence of depression, claustrophobia, psychiatric disorder, hypertension, diabetes mellitus, transient ischemic attacks or cardiovascular problems. A blood sample was also collected to determine their ApoE status. On the basis of this genetic profile the aMCI subjects were divided in two genotype categories:

- Category 1 (7 subjects heterozygous for the ApoE ε4 allele ε3ε4)
- Category 2 (11 subjects homozygous and heterozygous for the ApoE ε3 allele, ε3ε3/ε3ε2).

The aMCI subjects in category 1 had a mean MMSE score of 27.71 (SD = 2.75). The mean age of aMCI carriers was 67.86 years (SD = 6.0) with a mean education of 11.57 years (SD = 5.06). The aMCI subjects in category 2 had a mean Mini Mental State Examination (MMSE) score of 27.45 (SD = 2.38). The mean age of aMCI non-carriers was 70.82 years (SD = 4.77) with a mean education of 7.36 years (SD = 3.52). The control group had a mean MMSE score of 29.16 (SD = 1.04). The mean age of the controls was 62.25 years (SD = 5.83) with a mean education of 10.33 years (SD = 4.57). Local ethical committee approval was obtained and all MCI patients and controls gave written informed consent.

5.2.2.2 ***Neuropsychological Assessment***

Each of the MCI and control subjects completed a comprehensive neuropsychological assessment. The following battery of neuropsychological tests was administered; the Mini Mental State Examination (MMSE), verbal paired associates, confrontation naming; semantic and letter fluency task, forward and backward digit span, visual spatial span, visual-spatial supraspan learning, Raven's standard progressive matrices (PM47), token test, Rey complex figure task (direct and delayed copy) and the Stroop test (error and time interference effects). Table 5.1 summarises the neuropsychological profile obtained by the two MCI groups (carriers and non-carriers) and healthy controls.

Table 5.1. *Mean (SD) neuropsychological scores of MCI carriers, MCI non-carriers and Controls.*

Tests	MCI ε4 Carriers	MCI ε4 non Carriers	Controls
Mini Mental State Examination	27.71 (2.75)	27.45 (2.38)	29.00 (1.04)
Verbal paired associates	4.71 (1.35)	5.18 (1.19)	9.46 (4.30)
Confrontation naming	19.14 (0.69)	17.54 (2.80)	19.72 (0.47)
Letter fluency (no words)	19.85 (11.52)	23.00 (8.34)	30.90 (10.03)
Category fluency (no words)	29.71 (15.01)	27.90 (8.15)	39.90 (5.01)
Category fluency (AoA)	4.64 (1.21)	4.90 (0.62)	6.53 (0.96)
Digit Span:			
Forward	5.86 (0.69)	4.73 (0.65)	5.45 (1.37)
Backward	3.28 (1.70)	3.09 (0.83)	4.45 (1.36)
Visual-spatial span	3.71 (0.49)	4.00 (0.45)	4.54 (0.69)
Visual-spatial supraspan learning	1775 (6.81)	17.43 (8.46)	24.76 (4.23)
Raven's coloured progressive matrices (PM47)	26.57 (4.68)	26.18 (6.24)	31.00 (3.90)
Token test	33.42 (1.48)	32.05 (4.19)	34.14 (1.58)
Digit Cancellation	47.71 (6.24)	45.09 (11.30)	52.45 (6.42)
Rey complex figure:			
Direct copy	31.71 (3.09)	29.73 (7.88)	33.27 (2.69)
Delayed copy	12.07 (5.95)	11.27 (5.95)	17.05 (7.01)
Stroop test			
Error interference effect	1.07 (1.10)	3.45 (4.27)	0.18 (0.60)
Time interference effect	23.79 (10.24)	34.27 (15.67)	23.09 (11.67)

5.2.2.3 ***Experimental Procedure***

Lexical competency assessment

Each individual performed a semantic fluency task in order to assess their lexical competency. Two semantic categories were administered; animal and fruit. All the subjects were asked to produce as many words as possible belonging to these categories in two 60 second trials. The total number of words was recorded and the age of acquisition (AoA) value for each acceptable word was determined. The data included in the analyses were the mean attributional values for the words produced by each patient. Age of acquisition (AoA) values for words were taken from the rating collected for another study in a sample of normal elderly controls (see study III in Chapter 4). These controls were from a similar geographical and socio-cultural background as the patients and controls enrolled in this study. Ratings acquired in this way have been shown to correlate highly with objective measures of AoA and therefore have good validity (Morrison, et al., 1997).

Structural MRI scanning: acquisition and analysis

Three dimensional T1-weighted MRI images were acquired on a 3.0 T Philips Intera system with a Turbo Field Echo sequence. Voxel dimensions were 1.00 x 1.00 x 1.00 mm. Field of view was 256 mm with a matrix size of 256 x 256 x 124, TR 9.9 msec, TE 4.6 msec and flip angle 8°, total duration 4 minutes 41 seconds. A number of preprocessing steps were followed to isolate the grey matter (GM) from the 3D T1-weighted structural scans before performing the statistical analysis using SPM5 (Wellcome Department of Imaging Neuroscience, UCL, London, UK). To correct for global differences in brain shape, structural images were warped to standard stereotactic space and segmented to extract grey matter, white matter and cerebrospinal fluid. The grey matter segments were then modulated to correct for changes in volume induced by nonlinear normalisation and smoothed using a Gaussian filter set at 8 mm to reduce possible error from between-subject variability in local anatomy and render the data more normally distributed. Smoothed modulated grey matter segments were entered into the analyses. Independent t-tests were used for group comparisons. In addition GM segments were entered into a voxel-based multiple regression analysis to investigate linear correlations between GM concentration and retrieval of later acquired words. Finally, a conjuction analysis was carried out to look at the AoA and ApoE ε4 conjunct effect. Age, number of years of education, MMSE and gender were also included in all the analyses as covariates. The x,y,z coordinates of the areas of significant correlation obtained from the analyses were first converted into Talairach coordinates using the Matlab function mni2tal (http://imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mni2tal.m) and then identified using the Talairach Daemon Client (http://ric.uthscsa.edu/projects/tdc/). Unless otherwise specified height threshold was set at $p < 0.05$ uncorrected and a miminum extent threshold of 50 voxels was used for all analyses. An uncorrected height threshold was deemed acceptable, given the small sample size and based on a priori hypotheses which could be used to guide focus on specific regions of interest. A T2-weighted axial scan was also acquired prior to the 3D scan acquisition to better highlight the presence of any vascular lesions and to ensure that all participants included in the 3D structural imaging study had no significant vascular burden.

5.2.3 Results

The three groups did not differ in education ($F_{(2, 28)} = 2.67$, *ns*) and MMSE scores ($F_{(2, 28)} = 1.88$, *ns*) but were not matched for age [aMCI non carriers > aMCI carriers > control non carriers, ($F_{(2, 28)} = 8.39$, $p < .05$)].

5.2.3.1 ***Neuropsychological assessment***

An ANCOVA with age as a covariate was carried out among these groups (aMCI carriers, non-carriers and controls). There were no significant differences in any of the neuropsychological tests but one; in the verbal paired associates learning task the aMCI group (carriers and non-carriers) performed significantly worse than controls ($p < .001$), however no difference was found between aMCI carriers and aMCI non-carriers.

The Age of Acquisition data from the fluency task were analysed in detail (see Table 5.1). ANCOVA showed a significant difference among the three groups in lexical performance ($F_{(2, 28)} = 7.394$, $p < .05$). Post hoc Sheffe’ analysis showed a significant difference ($p = .01$) in the AoA values of the aMCI carriers and non-carrier compared to controls.

5.2.3.2 ***Voxel-based comparison analysis***

<u>Controls versus MCI carriers</u>

The direct grey matter volume comparison between non-carrier healthy control and aMCI ApoE ε4 carriers showed significant areas of atrophy in the left parahippocampal gyrus, posterior cingulate, precuneus, in the righ subcallosal gyrus and cuneus and in the thalamus, the caudate nucleus, the cerebellum and the lingual gyrus bilaterally. Significant areas of lower grey matter values were also found in the left middle and right inferior occipital gyrus, in the right middle, inferior and superior frontal gyrus and in the temporal gyrus bilaterally. Table 5.2 shows all the areas of significant lower grey matter volume in aMCI carriers.

Table 5.2. *Areas of smaller grey matter volume in carriers compared to controls.*

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates			Z-value at local maximum
				x	y	z	
Subcallosal Gyrus	R	25	2137*	6	5	-12	3.49
Caudate (head)	L			-4	10	-4	3.31
Parahippocampal Gyrus	L	34		-18	7	-15	2.89
Superior Frontal Gyrus	R	9	117	26	33	33	3.48
Thalamus (medio dorsal nucleus)	R		908	10	-21	12	3.23
Thalamus (pulvinar)	L			-8	-25	9	3.16
Caudate (body)	R			16	-11	19	2.81
Cerebellum (Culmen)	R		337	38	-44	-30	3.22
Cuneus	R	18	1011	4	-93	10	3.06
Middle Occipital Gyrus	L	18		-10	-93	12	2.67
Lingual Gyrus	R	17		6	-91	-4	2.55
Cerebellum	L		158	-36	-50	-31	2.77
Lingual Gyrus	L	19	92	-32	-60	-4	2.74
Inferior Occipital Gyrus	R	18	102	44	-80	-9	2.61
Superior Frontal Gyrus	R	8	143	20	28	48	2.55
Middle temporal Gyrus	R	19	83	40	-81	17	2.55
Precuneus	L	7	76	-18	-52	50	2.47
Superior Parietal Lobule	L	7		-24	-62	44	1.92
Posterior Cingulate	L	30	92	-12	-60	10	2.43
Middle Frontal Gyrus	R	10	116	40	56	-8	2.36
Superior Frontal Gyrus	R	10		30	56	-1	2.20
Middle Temporal Gyrus	L	37	88	-46	-58	1	2.27
Inferior Frontal Gyrus	R	11	51	16	26	-20	2.25
Middle Frotal Gyrus	R	11	59	34	470	-15	2.21
Insula	R	13	64	36	18	8	2.18
Superior Temporal Gyrus	R	38	51	36	22	-30	2.12

*p < .005

Controls versus aMCI non-carriers

When the comparison was carried out to look for grey matter volume differences between non-carriers healthy controls and aMCI non-carriers, areas of significant atrophy were detected in the right uncus, in the left parahippocampal gyrus, precuneus, caudate nucleus, the hippocampus, thalamus and cuneus, and in the lingual gyrus and fusiform gyrus and cerebellum bilaterally. Further areas of lower grey matter volume in aMCI and carriers were found in the middle and inferior frontal gyrus, in the middle, superior temporal gyrus bilaterally, in the left middle occipital gyrus, in the right rectal gyrus and in the left lentiform nucleus. Table 5.3 shows a detailed summary of all the areas of significant atrophy in aMCI non-carriers.

Table 5.3. *Areas of smaller grey matter volume in non-carriers compared toControls.*

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates x	y	z	Z-value at local maximum
Cuneus	R	18	137	26	-77	20	2.67
Cerebellum	R		1063	10	-46	-31	3.16
Cerebellum	L			-2	-54	-28	2.42
Inferior Frontal Gyrus	R	9	88	50	7	29	3.15
Thalamus (medio dorsal nucleus)			1797	-12	-21	12	2.93
Ligual Gyrus	R	18		26	-8	-9	2.67
Middle Temporal Gyrus	L	21	646	-42	1	-25	2.85
	L	37		-50	-38	-13	2.46
Parahippacapal Gyrus	L	36		-30	-26	-24	2.41
Middle Occipital Gyrus	L	18	52	-28	-80	-3	2.84
Lingual Gyrus	R	19	56	18	-58	1	2.79
Precuneus	L	31	88	-22	-73	26	2.74
Insula	L	13	169	-40	16	10	2.68
Precentral Gyrus	L	44		44	-80	-9	2.04
Middle Frontal Gyrus	R	9	60	40	23	30	2.55
	L	11	233	-40	38	-12	2.53
Inferior Frontal Gyrus	L	47		-18	-52	50	2.32
insula	R	13	182	38	-22	-9	2.50
Parahippocampal Gyrus	R	20		-12	-60	10	2.29
Fusiform Gyrus	L	37	97	-36	-39	-11	2.49
Lingual Gyrus	R	18		-34	-66	-2	2.16
Fusiform Gyrus	R	20	156	48	-32	17	2.43
		36		44	-36	-22	2.38
Middle Temporal Gyrus	R	20		51	-41	-11	1.96
	R	21	80	50	2	-35	2.39
Superior Temporal Gyrus	R	38		36	18	-35	2.13
Lentiform Nucleus	L		149	-22	-12	-6	2.30
Caudate	L			-32	-27	-4	2.13
Hippocampus	L			-36	-22	-9	2.01
Uncus	R	28	98	22	5	-22	2.29
Inferior Frontal Gyrus	R	11	114	18	26	-18	2.27
		47		26	23	-10	1.92
Rectal Gyrus	R	11		8	42	-24	1.87

<u>aMCI non-carriers versus aMCI carriers</u>

A direct comparison between aMCI carriers and aMCI non-carriers showed areas of significant lower grey matter volume in non-carriers in the left cingulate gyrus, precuneus and the superior and inferior temporal gyrus; in the cerebellum, middle occipital gyrus and superior, middle frontal gyrus bilaterally and in the right inferior frontal gyrus. Table 5.4 and Figure 5.1 show the areas of significantly smaller grey matter volume in aMCI non-carriers compared to aMCI carriers.

Table 5.4. *Areas of smaller grey matter volume in non-carriers versus carriers.*

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates			Z-value at local maximum
				x	y	z	
Middle Occipital Gyrus	R	19	7834*	30	-77	13	4.27
Inferior Frontal Gyrus	R	9		40	7	25	3.07
Precentral Gyrus	R	6		51	0	33	3.06
Superior Temporal Gyrus	L	13	7660*	-44	-45	23	3.79
Inferior Temporal Gyrus	L	20		-46	-4	-33	3.36
Superior Temporal Gyrus	L	38		- 26	14	-33	3.18
Medial Frontal Gyrus	R	10	5239*	14	49	9	3.18
Superior Frontal Gyrus	L	11		-12	53	-21	2.92
Medial Frontal Gyrus	R	9		12	40	24	2.89
Postcentral Gyrus	L	40	51	-40	-34	57	2.95
Cingulate Gyrus	L	31	201	-14	-55	25	2.53
Precuneus	L	7		-2	-62	33	1.86
Cerebellum	R		102	2	-45	-40	2.46
Medial Frontal Gyrus	L	46	123	-42	28	13	2.43
Paracentral Lobule	L	6	80	0	-28	62	2.31
Superior Frontal Gyrus	R	6	95	6	13	62	2.30
Medial Frontal Gyrus	R	6		4	-9	61	2.25
Superior Frontal Gyrus	L	6		0	5	61	1.74
Middle Occipitale Gyrus	L	19	75	-42	-74	2	2.29
Cerebellum(Culmen)	L		164	-4	-32	-12	2.27
Middle Frontal Gyrus	L	9	51	-46	29	26	2.20
Inferior Frontal Gyrus	R	47	62	48	-32	17	2.11
Middle Frontal Gyrus	L	46	93	48	16	-1	2.05
		10		-36	44	24	1.96

*p < .001

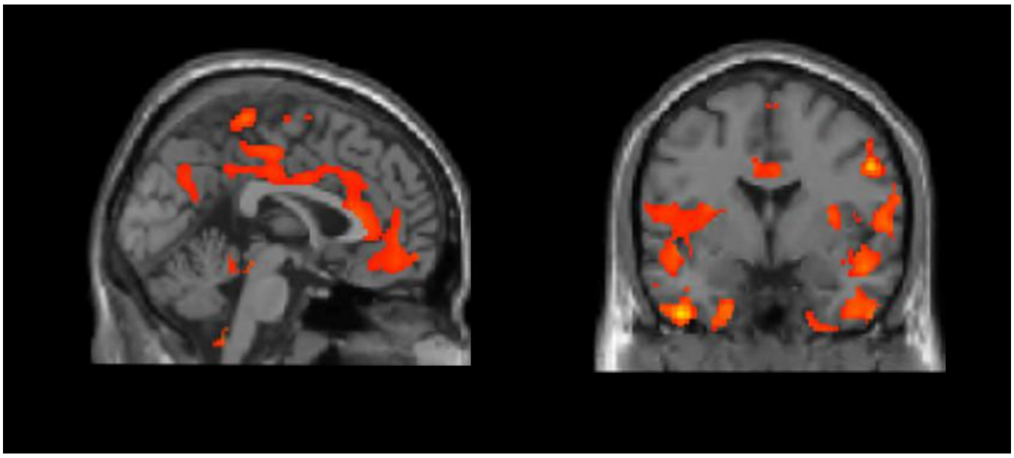


Figure 5.1. *Smaller grey matter volume in aMCI non-carriers compared to aMCI carriers*

aMCI carriers versus aMCI non-carriers

This analysis showed the areas of lower grey matter volume in aMCI carriers where compared to aMCI non-carriers. Significantly greater atrophy in carriers was detected in the left parahippocampal gyrus and uncus, in the caudate nucleus (head), in the cerebellum and superior middle and medial frontal gyrus and lentiform nucleus bilaterally, and in the right cuneus, fusiform gyrus and middle temporal gyrus. Table 5.5 and Figure 5.2 summarise the areas where significantly lower grey matter volume in aMCI carriers was found when compared to aMCI non-carriers.

Table 5.5. *Areas of smaller grey matter volume in carriers versus non-carriers*.

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates			Z-value at local maximum
				x	y	z	
Cerebellum	R		4487*	34	-52	-23	4.78
Middle Frontal Gyrus	R	6	485	28	4	50	4.08
Superior Frontal Gyrus	R	6		24	11	55	3.35
Middle Frontal Gyrus	R	8		28	16	45	3.21
Caduate	R		369	16	23	-6	3.86
Lentiform Nucleus	R			24	13	-6	2.59
Caudate	L		407	-16	21	-4	3.84
Lentiform Nucleus	L	11		-24	5	-7	2.48
Inferior Frontal Gyrus	L	47		12	40	24	2.37
Cerebellum (Culmen)	L		191	-20	-38	-20	3.52
Parahippocampal Gyrus	L	28		-22	-19	-21	2.08
	L	35		-22	-28	-17	1.86
Cuneus	R	18	151	18	-71	15	3.09
	R	17		10	-81	8	2.67
Middle Frontal Gyrus	L	6	52	-38	2	44	2.89
Superior Frontal Gyrus	L	9	55	-14	39	40	2.59
Medial Frontal Gyrus	L	8		-10	29	45	1.87
Precentral Gyrus	L	4	58	-14	-26	64	2.33
Medial Frontal Gyrus	L	6		-10	-26	57	1.88
Fusiform Gyrus)	R	37	74	46	-59	-9	2.22
Middle Temporal Gyrus	R	22		50	-49	-1	1.90
Inferior Frontal Gyrus	R	11	97	22	34	-17	2.21
Middle Frontal Gyrus	R	11		32	38	-14	1.99

*p < .001

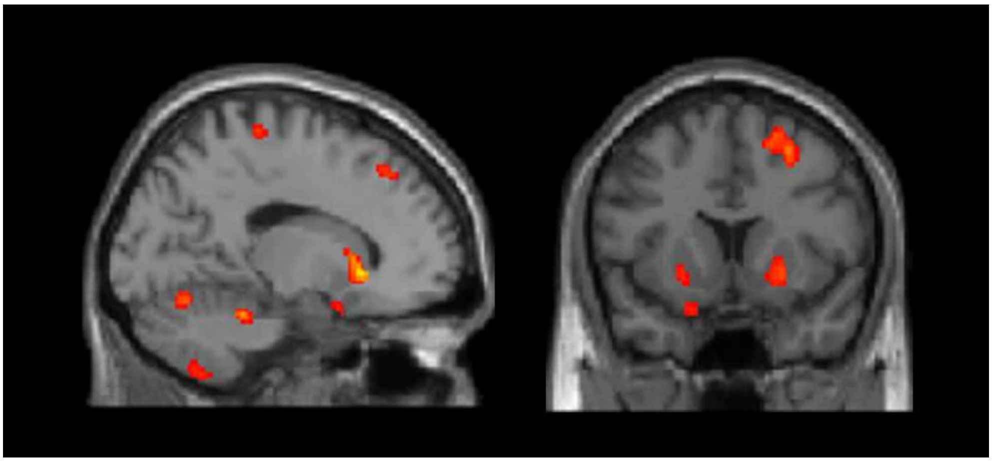


Figure 5.2. *Smaller grey matter volume in aMCI carriers compared to aMCI non-carriers.*

5.2.3.3 ***Voxel Based correlation analysis***

Multiple regression analyses were carried out to identify brain regions whose grey matter values significantly correlated with reduced retrieval of later acquired words. Age, years of education, MMSE score and gender were included in the model as covariates.

<u>AoA effect</u>

Residual later acquired words was correlated significantly with higher grey matter values in the parahippocampal gyrus, the middle temporal gyrus, superior, middle and medial frontal gyrus, and cerebellun bilaterally, in the right uncus, hippocampus and caudate nucleus, and in the left postcentral and supramarginal gyris. Table 5.6 and Figure 5.3 summarise the brain regions whose grey matter values significantly correlated with AoA values.

Table 5.6. *Areas in which GM volume values significantly correlated with age of acquisition word values*.

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates x	y	z	Z-value at local maximum
Middle Temporal Gyrus	L	37	64	-42	-56	-2	3.17
Cerebellum	R		111	14	-51	-46	3.09
Postcentral Gyrus	L	40	191	--40	-29	47	2.91
Middle Frontal Gyrus	L	8	79	-22	27	37	2.73
Superior Frontal Gyrus	L	9		-20	38	28	2.29
	L	10		-22	45	16	1.75
Parahippocampal Gyrus	L	36	97	-40	-32	-22	2.70
Fusiform Gyrus	R	20	127	44	-34	-18	2.57
Parahippocampal Gyrus	R	36		38	-28	-24	2.32
Uncus	R	34	161	20	3	-20	2.55
Superior Temporal Gyrus	R	38		38	5	-19	2.16
Parahippocampal Gyrus	R	34		12	-7	-18	1.95
Parahippocampal Gyrus	L	34	200	-16	-11	-18	2.50
Uncus	L	28		-26	3	-20	1.90
Cerebellum	L		132	-48	-68	-30	2.39
Medial Frontal Gyrus	R	9	61	20	35	31	2.39
Superior Frontal Gyrus	R	9		26	40	31	1.89
Middle Frontal Gyrus	R	9		34	33	35	1.82
Middle Temporal Gyrus	R	21	108	51	-28	-7	2.20
Middle Frontal Gyrus	L	9	53	40	-25	32	2.20
Precentral Gyrus	L	9		-42	19	38	1.78
Parahippocampal Gyrus	R	19	83	28	-43	-1	2.09
Caudate	R			32	-31	-0	1.88
Hippocampus	R			36	-24	-9	1.82

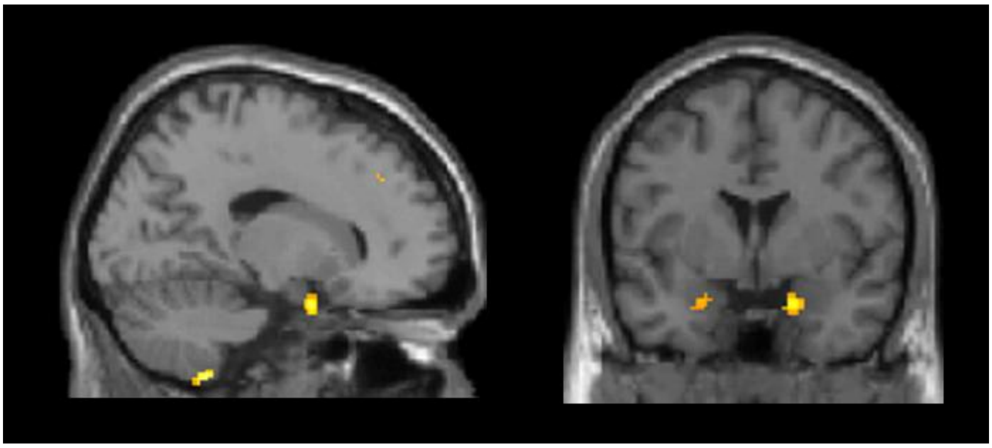


Figure 5.3. *Area of significant correlation between grey matter volume and age of acquisition values.*

5.2.3.4 ***Voxel based conjuction analysis***

A conjunction analysis was carried out to show regions of significant overlap between volume differences in aMCI carriers versus non-carriers healthy controls and areas of significant correlation of grey matter volume and AoA values. Regions of overlap were found in the left caudate nucleus, right parahippocampal gyrus and in the thalamus bilaterally. Table 5.7 and Figure 5.4 show the results of this analysis.

Table 5.7. *Areas of overlap between volume loss in carrier compared to controls and those where correlation with semantic retrieval was found*

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates			Z-value at local maximum
				x	y	z	
Caudate	L		4	-6	-10	-2	1.93
Thalamus	L		11	-8	-27	11	1.90
Parahippocampal Gyrus	R	34	2	8	1	-14	1.84
Thalamus	R		1	8	-23	-12	1.67

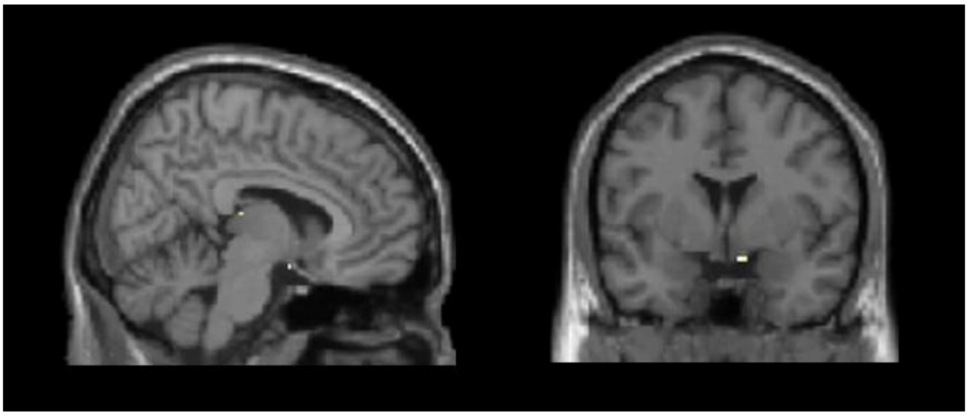


Figure 5.4. *Areas of overlap between smaller grey matter volume in aMCI carriers compared to controls and those areas where a significant correlation with AoA values was found.*

5.2.4 Conclusion

This study aimed to investigate grey matter differences in aMCI carriers/non-carriers when compared to healthy non-carrier controls and to clarify the interplay between ApoE ε4 burden in regions involved in semantic retrieval skills and the subtle lexical deficits seen in MCI patients carrying this mutation. The behavioural data show no differences between the aMCI as a whole group and controls in any of the neuropsychological test except for score on the verbal paired associates learning task ($p < .001$). Significant differences in lexical performance (AoA values) were also seen between the aMCI group as a whole and controls. However, although only detectable as a trend, MCI ε4 carriers showed major impoverishment of semantic retrieval abilities in the category fluency task when compared to aMCI non-carriers. The VBM analysis identified different atrophy patterns between the genetically determined subgroups based on the presence of the ApoE ε4 allele. In aMCI carriers, lower grey matter volumes were mainly identified in mediotemporal structures, the posterior cingulate and parietal cortex when compared to non-carrier controls. aMCI non-carriers, although showing a broadly similar atrophy pattern in mediotemporal regions, they also had lower grey matter volume in some areas of the neocortex bilaterally. This different atrophy pattern was more evident during the direct aMCI carrier/non-carriers comparison which revealed smaller grey matter volume in parietal regions and in the temporal lobe for the aMCI non-carriers and smaller grey matter volume in the parahippocampal gyrus for the aMCI carriers. Correlation analysis showed an association between poorer retrieval skills and atrophy of the left parietal, medial temporal and frontal regions and cerebellum bilaterally. Finally, a conjunction analysis of regions of significant overlap between volume differences in aMCI carriers versus non-carrier healthy controls and areas of significant correlation of grey matter volume and AoA values showed regions of overlap in the left caudate nucleus, right parahippocampal gyrus and thalamus bilaterally.

5.3 STUDY II: The relationship between ApoE ε4 genotype and brain volume in patients with minimal to mild AD

5.3.1 Introduction

Evidence indicates that brain changes in individuals at genetic risk for developing AD begin many years or decades before the onset of the disease. Neuroimaging techniques such as MRI, fMRI and several variety of PET imaging, show differences at the group level between ApoE ε4 carriers and non-carriers in the severity of the brain change in AD, MCI and healthy controls (see Chapter 2 sections 2.6.5, 2.6.6 and 2.6.7). Athough some studies have shown contradictory findings, most of them found greater mediotemporal vulnerability to the ε4 allele mutation across the different groups (Filippini, et al., 2009a; Pievani, et al., 2009; Burggren, et al., 2008; Mueller, et al., 2008; Reiman, et al., 2004; Thomann, et al., 2008; Pennanen, et al., 2006) . Cognitive deficits can also be seen at the preclinical stage of AD together with neuropathological changes, but to predict outcomes in those who have not yet developed the disease is a challenge mostly because of the lack of indicators which can consistently detect the disease. Supported by recent evidence which have shown that sophisticated methods of cognitive assessment associated with neuroimaging techniques can more accurately detect the distinction between normal and abnormanl cognitive decline (Venneri, et al., 2008), in the previous studies presented in this dissertation, it was found that lexical semantic deficits linked to greater mediotemporal volume loss might be used as a cognitive endophenotype of AD pathology in a group of aMCI individuals carriers of the ApoE ε4. However, to better clarify whether the conclusion about the specific deteriorating effect of ApoE ε4 burden on the brain structures mainly confined in areas involved in semantic retrieval from long term memory is consistent, the next study was carried out to investigate wheter the differential pattern of neuropathological spread of neuronal loss observed in the two genetically identified MCI subgroups was also detectable at a more advanced stage of the disease such that of mild to moderate AD.

5.3.2 Methods

5.3.2.1 *Participants*

Twenty nine patients with probable Alzheimer's disease of minimal to mild severity participated in this study. The patients underwent neuropsychiatric assessment, neurological examination and extensive neuropsychological screening. None of the patients in this group met the criteria for MCI (Petersen, et al., 1999) as they all had difficulties in activities of daily living and\or instrumental activities of daily living at the time of first referral. A blood sample was also collected in order to determine their ApoE status.

Two genotype category subgroups were found:

- Category 1 (patients homozygous and heterozygous for the ApoEε4 allele, ε4ε4\ ε3ε4) (N= 19, 8 homozygous and 11 heterozygous).
- Category 2 (patients homozygous and heterozygous for the ApoE ε3 allele, ε3ε3\ ε3ε2) (N=10, 8 homozygous and 2 heterozygous).

AD carriers had a mean Mini Mental State Examination (MMSE) score of 22.72 (SD = 2.86), AD non-carriers had a mean MMSE of 23.50 (SD = 3.17). The mean age of the carriers was 77.22 years (SD = 8.48) with a mean education of 9.61 years (SD = 1.14). The AD non-carriers had a mean age of 76.60 years (SD = 6.7) with a mean education of 13.20 years (SD = 3.42). All selected patients met the NINCDS-ADRDA criteria for a diagnosis of probable AD of mild severity, (Mckhann, et al., 1984) and none had radiological evidence of ischemic brain disease. Other causes of dementia were excluded according to published clinical criteria (McKeith, et al., 1996; Brun, et al., 1994; Roman, et al., 1993). All patients were re-assessed after at least six months from initial assessment and had their clinical diagnosis confirmed. The participants were all British and English was their first language. All the patients recruited for this study were right-handed.

5.3.2.2 ***Structural MRI scanning: acquisition and analysis***

Three-dimensional T1-weighted MRI images were acquired on a 1.5T GE NVi MRI system with an SPGR sequence. Voxel dimensions were 0.937 mm × 0.937 mm × 1.6 mm. Field of view was 240 mm with a matrix size of 256 × 256 × 124. Total acquisition time was seven minutes and twenty six seconds. A number of preprocessing steps were followed to isolate the grey matter (GM) from the 3D T1-weighted structural scans before performing the statistical analysis. The method of optimized voxel-based morphometry developed by Good et al., (2001) was implemented to improve segmentation. This involved the creation of a customised template using the structural MRI scans of the patients from the study. The initial T1-weighted images from the patients were normalised to the Montreal Neurological

Institute (MNI) template that was provided with SPM2 (Wellcome Department of Imaging Neuroscience, UCL, London, UK). The average of these normalised brains was taken to create the customised template. Customised grey matter priors for voxel classification were also made by segmenting the grey matter from the normalised images and creating an average from all the participants. Priors were also created for white matter (WM) and cerebro-spinal fluid (CSF). The initial T1 images were then segmented into grey matter, white matter and cerebro-spinal fluid (using the customised GM/WM/CSF templates as prior probability maps for tissue classification). The segmented grey matter images were then normalised to the customised GM templates. The normalisation parameters from this stage of the preprocessing were saved. Finally, the initial T1 images were then normalised by applying the newly acquired normalisation parameters. These normalised images were segmented once again using the customised grey matter templates. The grey matter images were smoothed with a 4 mm full width at half-maximum isotropic Gaussian kernel. This reduced between participant variability in the anatomy of the gyri and improved the normality of the distribution of the imaging data, both of which were important for the statistical analysis. Smoothed grey matter segments obtained with this procedure were entered into a voxel-based regression test comparison analysis using SPM2 along with age, education and MMSE score included as covariates. A height threshold of $p < 0.005$ was used.

5.3.3 Result

AD ε4 carrier and AD non-carrier groups did not differ for age ($t_{(26)} = .19$, *ns*) and mean MMSE score ($t_{(26)} = .66$, *ns*) but AD carriers showed a significant lower education compared to non carriers: 9.61 mean education for AD carriers vs 13.20 mean education for AD non-carriers ($t_{(26)} = 4.10$, $p < .001$).

5.3.3.1 *Voxel-based group comparison*

AD ε4 carriers vs AD non-carriers

This analysis showed the areas of lower grey matter volume in AD carriers where compared to AD non-carriers. Significantly greater atrophy in carriers was detected in the left precuneus, cuneus, posterior cingulate and superior frontal gyrus, and in the medial frontal gyrus bilaterally when compared to AD non-carriers (see Table 5.8and Figure 5.5).

Table 5.8. *Areas of lower grey matter density in AD carriers vs AD non-carriers.*

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates			Z-value at local maximum
				x	y	z	
Cuneus	L	19	28	-4	-78	37	2.97
		18	22	-4	-92	19	2.59
Medial Frontal Gyrus	R	11	22	4	53	-18	2.83
Caudate Nucleus	L		11	-8	19	-3	2.77
Medial Frontal Gyrus	L	10	13	-4	56	-1	2.75
Precuneus	L	7	24	-16	-70	42	2.70
Cerebellum	L		21	-4	-64	-2	2.58
Medial Frontal Gyrus	L	6	4	0	-24	66	2.56
	R	6	2	18	3	61	2.52
Thalamus	R		1	2	-11	13	2.47
Posterior Cingulate	L	30	7	-4	-54	6	2.44
Precuneus	L	31	4	-6	-49	36	2.42

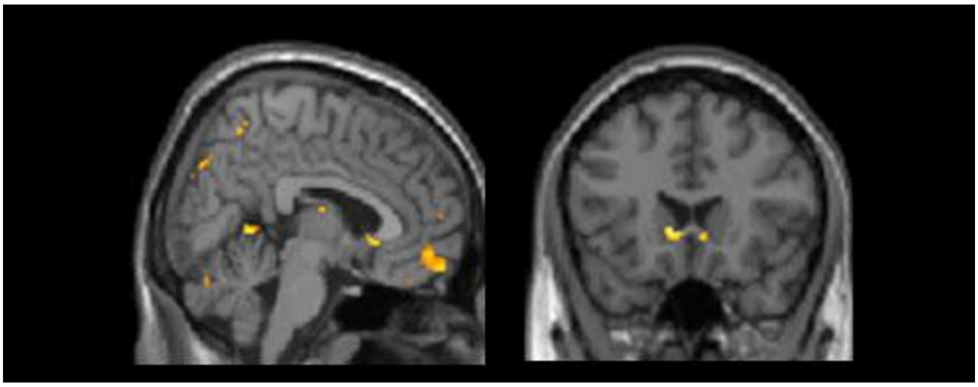


Figure 5.5. *Areas of smaller grey matter volume in AD carriers compared to AD non-carriers.*

<u>AD non-carriers vs AD ε4 carriers</u>

AD non-carriers had significantly lower grey volume values in extensive regions of the temporal, frontal and parietal neocortex bilaterally (see Table 5.9 and Figure 5.6). Additional smaller grey matter volume was found in the thalamus bilaterally, in the bilateral cerebellum, cuneus and posterior cingulate cortex.

Table 5.9. *Areas of lower grey matter density in AD ε4 non-carriers vs AD carriers.*

Brain area	Right/ Left	Broadmann's area	Cluster size	Talairach Co-ordinates x	y	z	Z-value at local maximum
Cerebellum	L		654*	-14	-66	-30	4.01
Precentral Gyrus	L	6	291**	-50	-1	13	3.98
Insula	L	13		-42	5	15	3.15
Middle Temporal Gyrus	L	21	38	-51	-24	-11	3.58
Middle Frontal Gyrus	R	6	76	34	6	42	3.43
Precentral Gyrus	R	6	136	50	2	37	3.32
Lentiform Nucleus	L		112	-14	8	-4	3.23
Middle Frontal Gyrus	L	6	59	-46	0	37	3.22
Superior Temporal Gyrus	R	22	28	61	-21	5	3.03
Thalamus	R		115	6	-11	4	3.00
Cerebellum	R		373	18	-64	-30	2.97
Middle Temporal Gyrus	R	21	23	48	4	-27	2.89
Superior Temporal Gyrus	L	22	13	-42	-52	17	2.85
Inferior Frontal Gyrus	R	46	52	46	31	8	2.84
	R	47		34	31	0	2.57
Superior Frontal Gyrus	R	6	8	12	16	53	2.80
Postcenral Gyrus	R	18	25	50	-31	35	2.79
Cuneus	R	18	9	14	-81	17	2.78
Middle Frontal Gyrus	L	9	13	-24	34	24	2.74
Supramarginal Gyrus	R	40	4	46	-39	31	2.71
Inferior Parietal Lobule	L	40	6	-36	-46	52	2.69
Inferior Frontal Gyrus	L	9	3	-34	11	23	2.65
	L	47	10	-28	29	-8	2.62
Precentral Gyrus	L	9	24	-40	19	36	2.60
Middle Frontal Gyrus	L	8		-32	17	38	2.51
Medial Frontal Gyrus	R	8	7	16	31	39	2.60
Thalamus	L		24	-4	-17	5	2.53
Inferior Temporal Gyrus	L	20	14	-50	-13	-28	2.51
Posterior Cingulate	L	29	3	-8	-46	15	2.46
Sub-Gyral	R	8	3	18	24	43	2.44
Inferior Parietal Lobule	L	40	1	-42	-49	36	2.43
Superior Temporal Gyrus	L	22	8	-61	-32	11	2.43
Sub-Gyral	L	37	7	-53	-39	-5	2.42
Superior Frontal Gyrus	L	10	1	-20	50	-1	2.40
Middle Frontal Gyrus	R	9	2	30	27	32	2.38
Supramarginal Gyrus	L	40	1	-40	-47	34	2.37
Middle Temporal Gyrus	L	37	2	-44	-55	-2	2.37

* $p < .005$; ** $p < .001$

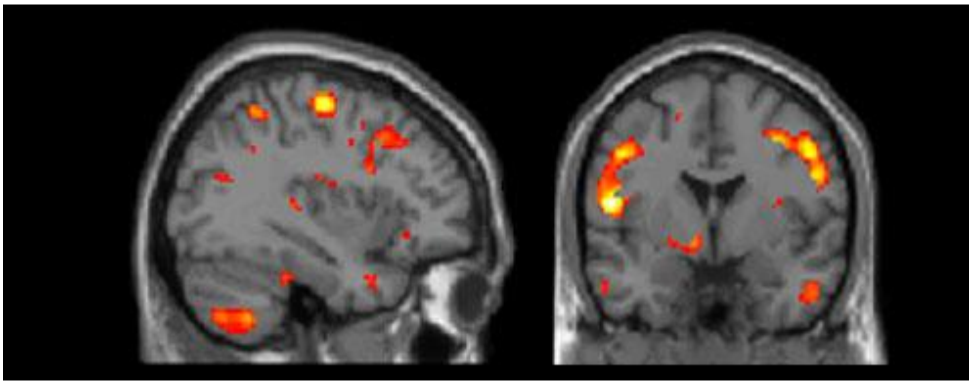


Figure 5.6. *Areas of smaller grey matter volume in AD ε4 non-carriers compared to AD carriers.*

5.3.4 Conclusion

This study aimed to investigate whether the burden of the ApoE ε4 had an effect on the topographical spread of grey matter volume loss in mild to moderate AD carriers and non-carrier of the ε4 mutation. The purpose was to verify if the detrimental role of this mutation found in aMCI individuals was consistent across groups characterised by having impoverished semantic retrieval skills and was maintained at a later stage in the disease process. VBM comparison analysis confirmed the observations in the genetically determined aMCI subgroups. AD carriers showed greater grey volume loss in mediotemporal structures compared to AD non-carriers whose atrophy was instead more widespread in more neocortical region

5.4 Overall discussion

Various studies have already shown the efficacy of the VBM technique to evaluate correlations between cognitive performance and local brain morphology in aMCI (Schmidt-Wilcke, Poljansky, Hierlmeier, Hausner, & Ibach, 2009; Barbeau, Ranjeva, Didic, Confort-Gouny, Felician, et al., 2008; Thomann, et al., 2008; Hamalainen, Tervo, Grau-Olivares, Niskanen, Pennanen, et al., 2007a; Chetelat, et al., 2003) and in AD (Di Paola, Macaluso, Carlesimo, Tomaiuolo, Worsley, et al., 2007; Berlingeri, Bottini, Basilico, Silani, Zanardi, et al., 2006). However, our study is the first to have investigated the effect of conjuncting a genetic mutation and specific lexical semantic deficits together as a unique endophenotype at the preclinical stage of Alzheimer's disease.

In the first study we examined MRI scans from 30 participants (7 aMCI carriers, 11 aMCI non-carriers and 12 non-carrier controls) to a) characterise grey matter volume differences between the three groups, b) detect anatomical features associated with impaired semantic retrieval and c) identify areas of overlap between grey matter volume group (aMCI carriers versus controls) differences and clusters associated with poorer semantic retrieval skills. Several interesting conclusions can be drawn from the obtained results. There were similar patterns of atrophy in both aMCI ε4 non-carrier and aMCI ε4 carrier groups when compared to healthy non-carriers, with greater involvement of medial temporal structures, precuneus and posterior cingulate cortex for the epsilon 4 carriers. The direct aMCI ε4 carriers/non-carrier comparisons highlighted a more evident and specific atrophy of mediotemporal regions (parahippocampal gyrus) in the aMCI ε4 carriers but greater presence of smaller neocortical grey matter volume (in particular temporal lobe and precuneus) for the aMCI ε4 non-carriers. At the cognitive level aMCI ε4 carriers and non-carriers, as a group, performed worse in all the neurospychological tests compared to controls including impoverished semantic retrieval abilities in the category fluency task. However, ε4 carriers showed the most impoverished output of all. It could be that these grey matter volume differences within the two aMCI groups could explain the different degree of semantic deficit observed in the aMCI ε4 carriers and non-carriers when compared to controls. The findings from the correlation and conjunction analyses seem to corroborate this hypothesis. Poorer retrieval skills resulting in production of earlier acquired words are associated with atrophy in the left parietal regions and in the medial temporal and frontal regions, and cerebellum bilaterally. The conjunction analysis, however, underlines the role of parahippocampal gyrus, caudate nucleus and thalamus as areas highly involved in poor semantic retrieval skills when associated with the presence of the ApoE ε4 mutation.

Few VBM studies have assessed brain morphology in MCI patients. The most common findings are decreased grey matter volumes in the medial temporal regions, posterior and neocortical part of the temporal lobes, posterior cingulated cortex, precuneus and frontal areas (Stoub, et al., 2006; Whitwell, et al., 2008; Chetelat, et al., 2005) . Smaller grey matter density in medial temporal regions in aMCI ε4 carriers compared to non-carriers has been found (Thomann, et al., 2008) and accelerated atrophy in ε4 carriers mainly in the hippocampus has also been reported (Morra, Tu, Apostolova, Green, Avedissian, et al., 2009). Moreover, after neuroimaging studies have shown areas of atrophy in aMCI ε4 carriers compared to non-carriers in medial temporal regions, with greater atrophy in the parahippocampal gyrus, amygdala and thalamus (Pennanen, et al., 2006), and abnormal activation in the thalamus and medial temporal structures during verbal paired associates learning in asymptomatic individuals with family history of AD (Bassett, et al., 2006). The morphometric data, therefore, confirm a selective genetically determined anatomic impact of the ε4 mutation on brain atrophy of people at the preclinical stage of AD. This observation is also supported by the findings of the second study which shows a similar pattern of volume differences in AD ε4 carriers compared to non-carriers. All these findings lead to the suggestion of a modulating and specific impact of the ϵ4 allele on the anatomical expression of the disease.

Another interesting point that comes from these studies is the relationship between the specific ε4 effect on MCI and AD brain volume and the different degree of semantic impairment demonstrated across these groups. A large body of evidence shows linguistic deficits in mild AD, in mild cognitive impairment subjects of amnestic type who will convert to AD and in people with a genetic mutation for familial AD (Risacher, et al., 2009; Adlam, et al., 2006; Arango-Lasprilla, et al., 2006; Duong, et al., 2006; Holmes, Fitch, et al., 2006; Forbes-McKay & Venneri, 2005). Imaging studies with fronto-temporal dementia and Alzheimer's disease, moreover, reported a relationship between volume value mediotemporal and temporal neocortical regions and semantic fluency performance (Birn, Kenworthy, Case, Caravella, Jones, et al.; Venneri, et al., 2008).

The greater atrophy involvement of the mediotemporal regions in ε4 carriers could be linked to the greater semantic deficits in subjects carrying this mutation. The influence of neocortical atrophy, instead, might be involved in the less severe but still present semantic deficit in aMCI non-carriers when compared to controls. It might be, that people with this mutation don't need extensive neocortical damage to show lexical semantic deficits, suggesting the presence of a less structured compensatory network compared to aMCI non-carriers whose more extensive neocortical atrophy doesn't seem to cause cognitive deficits to the same extent seen in aMCI carriers. This hypothesis seems to be supported by fMRI findings which showed greater signal change during semantic memory task in MCI and high risk groups than controls in medial temporal regions, area of the temporo/parietal junction and posterior cingulate/precuneus with a greater involvement of medial temporal structures for ε4 carriers (Woodard, et al., 2009).

It seems, therefore, that the early and greater semantic deficit observed in MCI carriers compared to non-carriers might be due to the greater neuropathological burden associated with the presence of the ApoE ε4 allele influencing those structures necessary for efficient semantic retrieval from long term memory. The presence of semantic deficits in MCI non-carriers is associated with greater neocortical deterioration and highlights the additional role of these structures in performing semantic memory tasks.

CHAPTER 6 A normative study of lexical semantic parameters

6.1 Introduction

Dementia and associated disorders in the elderly are expected to become one of the most public health problems (Ferri, Prince, Brayne, Brodaty, Fratiglioni, et al., 2005).There is, therefore, a need for reliable and valid neuropsychological instruments sensitive to the earliest signs of abnormal cognitive decline which might lead to dementia. One of the main issues in research and clinical settings is how to detect subtle cognitive deficits in a way that may discriminate normal cognitive function from preclinical cognitive markers which may characterise an intermediate stage between minimal variation of scores and significant abnormality that might be indicative of dementia (Petersen, Stevens, Ganguli, Tangalos, Cummings, et al., 2001). Detecting cognitive decline is a prerequisite in the diagnosis of dementia and mild cognitive impairment in order to plan therapeutic intervention and long-term care for patients and caregiver. To be useful measures and be able to track cognitive change over time, psychometric instruments or parameters have to be sensitive, specific, reliable and valid measures of the cognitive domains they are evaluating (Stein, Luppa, Brahler, Konig, & Riedel-Heller, 2010). In particular, the accuracy of diagnostic classification to some degree depends on their sensitivity (that is the probability in detecting abnormal functioning in an impaired individual) and specificity (that is, the probability of individuating a normal individual from another clinical population). The percentage of cases classified as accurately by any given test, however, will depend on the base rate of the conditions (population, demographic variable) used to evaluate its efficacy Most of the neuropsychological measures in the British population have not been standardised, adopting psychometric techniques of establishing inferential cut-offs based on large population samples (Bizzozero, Costato, Sala, Papagno, Spinnler, et al., 2000). It is well known that performance in cognitive tests can be influenced by confound and demographic variables including age, education and gender. Past research has provided ample evidence that performance on neuropsychological tests is heavily affected by demographic variables. Several studies have found, for example, that MMSE scores are significantly affected by age and education (Harvan & Cotter, 2006) that the short version of Raven's progressive matrices 1938 and the Stroop Test were significantly affected by age and education (Caffarra, Vezzadini, Dieci, Zonato, & Venneri, 2002; Caffarra, Vezzadini, Zonato, Copelli, & Venneri, 2003). Age-associated performance decrements and sex biases are seen on the CVLT (Delis, 1991), WMS-III (Ivnik, 1991), logical memory and paired associate word learning tests (Mitrushina, 1999), the recall trials on the complex figure test (Fastenau, Denburg, & Hufford, 1999) and on the token test (Spreen, 1998) . Education has been found to affect performance on vocabulary much more than age, particularly for older people who tend to have lower education levels (Kaufman, McLean, & Reynolds, 1988). The Verbal Fluency task is influenced by age (particularly for people over 70) with a positive age effect on semantics (Troyer, 2000), and no age, sex and education differences on letter fluency performance (Hughes & Bryan, 2002). In this dissertation and in previous recent studies lexical parameters such as Age of acquisition, typicality and familiarity are emerging as potentialy useful indicators of abnormal cognitive decline. The indicators coming from research studies do not find a ready application in clinical settings since there are quite clear indicators that ratings obtained from healthy participants vary extremely depending on demographic variables. Although there is evidence that self rating of items for AoA have a good agreement with children's real age of acquisition (C.M. Morrison, Chappell, & Ellis, 1997) these studies have noted large variation across gender, age classes and education levels. Based on these considerations and supported by the significant results of the study positing lexical semantic characteristics as possible cognitive endophenotype to be used in preclinical dementia diagnosis, the following logical step is to create a suitable database instrument of these parameters which can provide reliable and valid measures to identify subtle semantic deficits with particular interest to age, education and gender effect on age of acquisition, typicality and familiarity.

The age at which words are acquired has been considered an important issue in determining word processing efficiency in adulthood. Children start learning words around the age of 12 months, with a different rate of learned words per day. This process continues up to and through adulthood with an adult having a vocabulary of at least 20,000 words and an educated adult may know 70,000 words or more (Ingram, 1989;Oldfield, 1966). Earlier acquired words are highly correlated with the frequency of words in adult spoken language, with length (shorter words acquired earlier) and imageability (more concrete words tend to be learned earlier) (Goodman, Dale, & Li, 2008). These characteristics make it possible to speak about a degree of commonality in the way children build up their vocabulary and work out an average "age of acquisition (AoA)" that can be applied to the speaker belonging to the same language. Interestingly, Carroll & White (1973) discovered that adults are quite good at estimating the age of acquisition of words, finding a strong correlation between this measure and the one based on children's reading vocabularies, showing that Age of Acquisition is associated with faster naming, higher frequency and faster retrieval. A recent study has confirmed the findings of the Carroll and White study (Johnston, 2006). In general, faster processing of early words can be seen in comprehension (lexical decision task) and in production (speaking, reading and writing task) (Menenti & Burani, 2007; Hernandez-Munoz, 2006; Johnston, 2006). These studies found that the effect of age of acquisition didn't diminish with age; older participants were as strongly influenced by the age of acquisition as were the younger participants. This is an important point because if AoA was a reflection of the cumulative frequency of words these effects should diminish with age, but it doesn't, supporting the idea of something about early acquisition that makes for superior lexical representations in the mind and brain which remain easier to access and use during the whole life (De Deyne & Storms, 2007; Barry, 2006). In this regards researchers have investigated vocabulary loss in a wide range of neuropsychological disorders in an attempt to relate the age of acquisition effect with other cognitive-linguistic processes that are relatively spared or with the regions of the brain damaged in order to clarify and test hypothesis that earlier acquired words might have a superior lexical representation and the role of different regions involved in different linguistic tasks. Given its importance as a determinant of normal word retrieval speed in normal ageing, lots of studies have investigated if and how age of acquisition also affects word use in neuropsychological patients. Several studies showed that aphasic patients' residual language shows an effect of age of acquisition and probably also word frequency in object naming (Kittredge, Dell, Verkuilen, & Schwartz, 2008; Cuetos, Aguado, Izura, & Ellis, 2002). Overall, however, findings are confusing and contradictory, mainly because of the individual differences between aphasic patients, which mean that different kinds of factors affect differently each patient. Wollams et al., (2008) found less confusing data exploring naming performance in semantic dementia. In their study, age of acquisition, frequency and familiarity significantly affected naming accuracy. More consistent evidence comes from studies which have investigated the lexical effects in Alzheimer's disease patients. As already mentioned in previous chapters, progressive word-finding problems are a common feature in these patients. The main underliying cause of these problems seems to be related to damage to the core semantic representations of the meaning of the words and objects (Garrard, Perry, & Hodges, 1997). Some findings come from studies which have investigated lexical effects during naming of objects. Age of acquisition was found to significantly affect naming accuracy with better performance on early than late acquired words despite a non-significant effect of familiarity, word frequency and length (Silveri, Cappa, Mariotti, & Puopolo, 2002). Moreover, Holmes et al.,(Holmes, Fitch, & Ellis, 2006) suggested that this effect might be due to both a partial failure to recognise a depicted object as familiar and to the inability to call to mind the name of a recognised object. However, in that study, the authors also found that access to semantic knowledge (e.g. picture identification and naming) was influenced by the typicality of category exemplars. Typical exemplars which share similar features to one another (e.g. fox and lion) and the category prototype (animal) are named faster than those atypical examples (e.g. kangaroo and snake).

Other authors investigated to what extent the naming problems in Alzheimer's disease are different from the naming problems of normal adults. They found a similar profile of naming problems in healthy controls, albeit with much better overall performance, positing that the naming difficulties in Alzheimer's disease inherits the age of acquisition effect seen in normal adults (Newman & German, 2005;Hodgson & Ellis, 1998; C. M. Morrison, Hirsh, & Duggan, 2003). However, the difference in the magnitude of the age of acquisition effects in patients and healthy adults may be useful in diagnosis (Gale, Irvine, Laws, & Ferrissey, 2009). Other supporting data come from a study carried out by Forbes-McKay et al., 2005) who showed that the average age of acquisition of words generated was a good predictor of whether someone came from the Alzheimer's or control group. In particular, it was possible to classify correctly 88% of the Alzheimer patients and 95% of the controls by simply looking at the mean age of acquisition of the animals and fruits they generated. None of the other lexical characteristic of the words investigated in this study (typicality, mean number, length or frequency of the item produced) achieved the same level of discrimination. Interesting data also comes from a follow-up study, which investigated how lexical factors affect naming accuracy in Spanish-speaking patients with probable Alzheimer's disease (Cuetos, Rosci, Laiacona, & Capitani, 2008). These authors found an effect of age of acquisition but not word frequency or semantic category both in the first test and two years later. The items to which patients were unable to attempt any response had the latest average age of acquisition. In one of the few neuroimaging studies looking at the modulation of brain activity by age of acquisition, Ellis et al., (2006), using fMRI with healthy young people, found stronger activation for early than late items in the left temporal lobe, apparently attributable to the stronger activation of the semantics of early compared with the later acquired objects. Furthermore, Venneri et al., (2008) using a voxel based morphometry (VBM) identified areas of grey matter volume in the brain of Alzheimer's patients where loss of cortical tissue was most strongly associated with the effect of age of acquisition and other lexical factors. As reported previously they found significant correlations between grey matter density value in mediotemporal regions and age of acquisition value and typicality of words. Retention of cortical tissue in those areas was associated with better preservation of late vocabulary in Alzheimer's patients. Furthermore other recent studies have detected lexical semantic retrieval deficits in patients with MCI and in patients with heritable traits for early cognitive decline (for a review see Chapter 3 section 3.2.3). Finally, the cross-selectional AD and aMCI studies (see chapter 4 and 5) showed a) semantic deficit in people at preclinical stage of AD, with age of acquisition being the most robust lexical factor in discriminating MCI carriers from MCI non-carriers and healthy controls; b) an effect of the ApoE ε4 mutation revealing different atrophy pattern in ε4 carriers and non-carriers; c) substantial overlap between those areas which are subject to atrophy early in the course of the disease and those involved in semantic abilities (limbic areas) supporting the role of semantic lexical decline and in particular the age of acquisition effect, as possible preclinical endophenotype associated with brain atrophy in abnormal ageing.

The previous results, therefore, seem to confirm the efficacy of lexical semantic retrieval ability as a cognitive endophenotype at the preclinical stage of AD. However, in order to build up a valid and reliable cognitive marker, it is necessary to investigate how and whether demographic variables can affect individuals' rating performance and therefore misguide performance about individual patient's word retrieval performance.

The present chapter has three main objectives. Firstly, to present AoA, Familiarity and Typicality rating for 476 words (366 animals and 110 fruits) in English that might serve to aid future research on language deficits associated to AD. Secondly, to present regression analyses with age, gender and education as independent variables and age of acquisition, typicality and familiarity as dependent ones, to investigate the possible confounding effects of these demographic variables already seen to influence the performance during neuropsychological assessment.

Thirdly, to present a database of all the dimensions analysed for future use in research.

6.2 Method

6.2.1 Word sample

A total of 476 words (366 animals and 110 fruits) were obtained from an animal/fruit category fluency task performed by healthy participants. The sample includes both words referred to main categories (*bird, dog, fish, apple,* etc) and words referred to subcategories (*robin, Yorkshire terrier; clown fish, bramble,* etc).

6.2.2 Participants

One-hundred-fifty-one healthy volunteers were recruited for this study. All participants were native English speakers. Seven age categories were created to split up the sample according to the subject ages. The distribution of subject by age categories and education categories is shown in Table 6.1.

Table 6.1. *Demographic distribution of the study group. Values are total number of subjects (women/men)*

Education, years	Age group, years							Total
	18-20	21-30	31-40	41-50	51-60	61-70	>70	
≤ 11	1f	3f	2m	4(2f/2m)	3m	6(4f/2m)	5(3f/2m)	24
12-17	27(14f/13m)	23(14f/9m)	9(6f/3m)	4(1f/3m)	9(7f/2m)	7(5f/2m)	7(3f/4m)	86
≥ 17	3(1f/2m)	8(1f/7m)	9(5f/4m)	6(3f/3m)	6(2f/4m)	6(1f/5m)	3(2f/1m)	41
Total	31	34	20	14	18	19	15	151

6.2.3 Ratings procedures

For age of acquisition, familiarity and typicality ratings, the following procedure was used: the words were printed (font: Times News Roman, size 26), presented in a random order at the centre of the page, and alongside each word was a blank rating box. The pages were shuffled and assembled into booklets, so that each booklet contained the pages in a different random order. Due to the three different lexical attributes of the words used, the booklet was divided in three sections, each containing 366 animal and 110 fruit words. The order of presentation of each section to each participant was randomised using a Latin square procedure. The three section booklet was then administered to the subjects, who were asked to rate each word according to the instructions appropriate to the attribute considered. Participants were tested individually and they were given a consent form to sign and written instructions. This study received the approval of the local Regional Ethic Committee.

Age of acquisition

The instruction for the age of acquisition rating task were taken from another study in a sample of normal elderly controls (Forbes-McKay, et al., 2005). People were asked to indicate at what age they thought they had acquired a word by writing the age in the box next to each word. For example" If you think you have acquired the word "apple" when you were 3, just write 3 in the box on the left of the word "apple"; same thing for the animal category. If you don't know some animal or fruit names just leave the box blank and go ahead and continuing the task."

Typicality

Based on the instructions given by Larochelle, Richard and Souliers (2000), participants were asked to rate how well each exemplar (e.g. apple) represented its specific category (e.g. fruit). They were requested to rate the typicality of each item by using a 7-point Likert type rating scale, from 7 (most typical) to 1 (least typical). For example, “If you think Dolphin could be a better example of the animal category than Seahorse then you should choose a lower rating number for Seahorse (for example 3) and a higher ranting number for Dolphin (for example 6)”.

Familiarity

Familiarity ratings were obtained using instructions similar to those of Marques (Marques, Fonseca, Morais, & Pinto, 2007) but using a 7 point scale instead of 5 point scale. Raters were given a list of items split in two categories (animals and fruit). They were given a 7-point rating scale, from 7 (very familiar) to 1 (little familiar). They were asked to rate how familiar/unfamiliar they were with a particular item. How often they think about, or come into contact with that particular concept.

6.3 Results

This study collected norms for the lexical semantic parameters in a sample of 151 individuals from 18 to 92 years of age, evenly distributed across sex, age and education levels. In the analyses only the words rated from more than 80% of the individuals were used, respectively 357 out to 476 words for Age of Acquisition; 405 out 476 for typicality and 418 out to 476 for Familiarity.

Multiple regression analyses were carried out to investigate the effect of the demographic variables for each word rated by each individual for each lexical factor. Significant different load of the three variables for age of acquisition, typicality and familiarity values across the two categories (animal and fruit) were observed. The regression outcome for each word was examined and when the main effect was significant, β and t values for gender, age and education examined. The effect of each variable (if any) on rating performance is represented with the following symbols; #, *, ~, next to each animal and fruit word (see appendices).

Results show Age of Acquisition rating for words were the mostly affected by the demographic variables as a whole (269 out to 357 words), with gender affecting the most as compared to age and education (respectively 179 words vs 34 vs 56). Typicality and Familiarity rating were affected to a lesser degree, with gender mostly affecting familiarity ratings and age typicality ratings (see Table 6.2 below).

Table 6.2. *Number of words affected by demographic variables across lexical factors.*

	Gender	**Age**	**Education**	**Total**
AoA	**179**	34	56	269
Typicality	37	**60**	14	111
Familiarity	**99**	17	5	121

Mean AoA, Typicality and Familiarity ratings, their standard deviation and their range values are presented in the full database by alphabetic order. Age, Gender and Education effect for each word, if any, are also included (see Appendices A, B, C).

To evaluate the relation of AoA, typicality and familiarity rating mean values with the demographic variables, the whole sample was split up according to gender, age and education. For gender across the three lexical parameters no significant effects were found for any of the lexical parameters. t-tests were carried out showing no significant differences between males and females in AoA rating scores ($t_{(146)}$ = .39, p = ns); typicality rating score ($t_{(147)}$ = .24. p = ns) and familiarity rating score ($t_{(147)}$ = .77, p = ns) (see Tables 6.3, 6.4, 6.5 below)

Table 6.3. *Male and female AoA mean values.*

Gender	Mean (SD)	range (min-max)
Male	6.29 (2.23)	(1-78)
Female	6.58 (2.99)	(1-81)

Table 6.4. *Male and female Typicality mean values.*

Gender	Mean (SD)	range (min-max)
Male	3.06 (1.02)	(1-7)
Female	3.10 (1.01)	(1-7)

Table 6.5. *Male and female Familiarity mean values.*

Gender	Mean (SD)	range (min-max)
Male	2.98 (1.19)	(1-7)
Female	2.83 (1.16)	(1-7)

Education was also investigated across the linguistic parameters. A Univariate ANOVA was carried out to compare the mean values produced by each subjects across the three education categories (≤ 11; 12-16; ≥ 17) for each characteristic of the word. The result showed no significant difference for any AoA mean values ($F_{(2,145)} = 2.44$, p = ns), neither for typicality ($F_{(2,146)} = .59$, p = ns) or familiarity ($F_{(2,146)} = 2.89$, p = ns) (see Figures 6.1, 6.2, and 6.3 below).

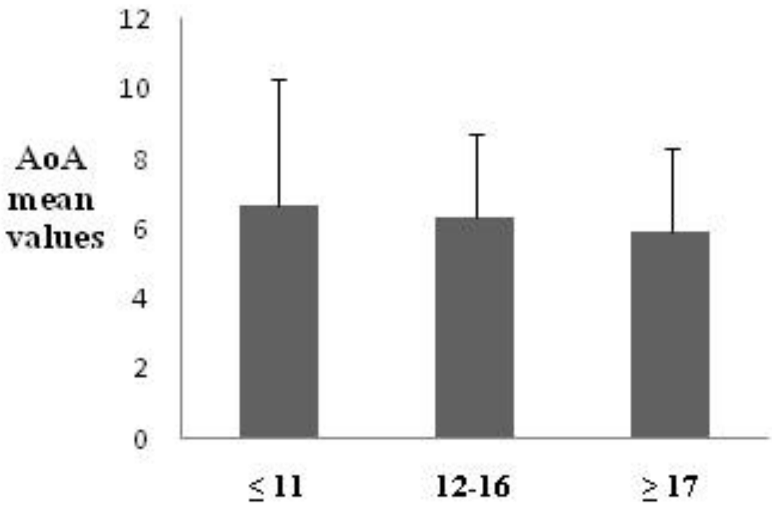


Figure 6.1. *AoA mean values for each of the three education levels.*

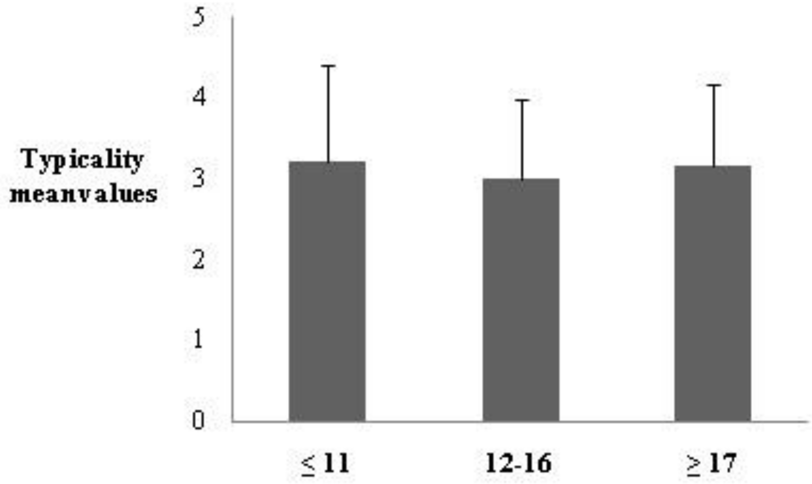


Figure 6.2. *Typicality mean values for each of the three education levels.*

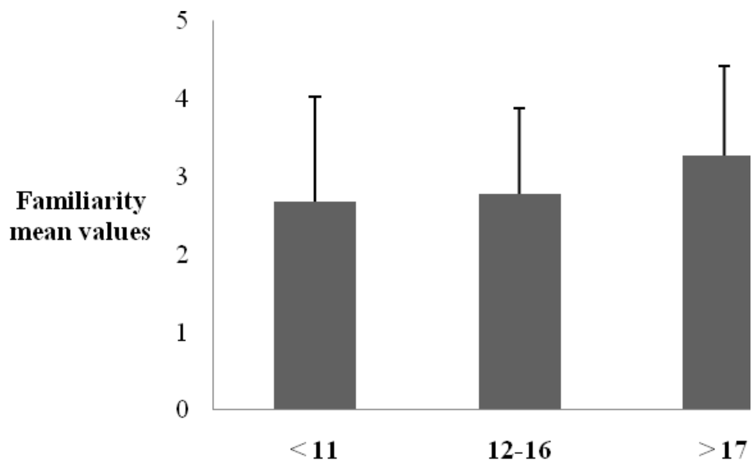


Figure 6.3. *Familiarity mean values for each of the three education levels.*

Finally, a Univariate ANOVA was carried out to see whether the age of individuals could significantly affect the lexical rating. Descriptive analysis showed that people belonging to the 21-30/31- 40 age categories obtained the least AoA mean values [respectively 6.13 (SD = .26) and 6.02 (SD = .34)] and people belonging to the 51-60/61-70 age categories get the highest AoA mean score [(respectively 7.59 (SD = .36) and 7.34 (SD = .36)]. Results showed that these age categories differences were significant ($F_{(6,141)} = 3.106$, $p = .007$). Post hoc analysis, using Bonferroni test, showed that people belonging to the 21-30 and 31-40 age categories rated words as acquired significantly earlier than people in the 51-60 categories (respectively p = . 026 and p = .038). Table 6.6 summarises the finding.

Table 6.6. *AoA mean (SD) and range (min-max) values across different age categories.*

Age range	Mean (SD)	range (min-max)
18-20	6.45 (1.17)	1-20
21-30	6.14 (1.17)	1-29
31-40	6.03 (1.50)	1-40
41-50	6.77 (1.46)	1-44
51-60	7.59 (1.92)	1-60
61-70	7.34 (2.03)	1-60
>70	6.74 (1.68)	1-81

No significant differences were seen when the same analysis was carried out for Typicality and Familiarity rating as dependent variables.

Table 6.7. *Mean Typicality values for each age category.*

Age range	mean (SD)	range (min-max)
18-20	2.82(0.80)	1-7
21-30	3.01(.093)	1-7
31-40	3.09(0.95)	1-7
41-50	3.33(1.28)	1-7
51-60	3.33(1.15)	1-7
61-70	3.01(0.89)	1-7
>70	3.37(1.38)	1-7

Table 6.8. *Mean Familiarity values for each age category.*

Age range	mean (SD)	range (min-max)
18-20	2.57(0.93)	1-7
21-30	2.97(1.12)	1-7
31-40	2.84(0.96)	1-7
41-50	3.64(1.40)	1-7
51-60	3.14(1.17)	1-7
61-70	2.86(1.42)	1-7
>70	2.59(1.25)	1-7

6.4 Discussion

This study aimed to collect normative data for age of acquisition, typicality and familiarity mean values in a large English population sample with an age raging from 18 to 92 and to evaluate the effect of age, education and gender on individual's rating performance. The full database of AoA, typicality and familiarity is provided as an appendix where the words (animal and fruit), listed alphabetically, are associated with the mean score and to a symbol indicating whether any of the demographic variables of interest had a significant influence on people's rating.

Regression models for each word showed a significant effect of the three demographic variables on each lexical characteristic of the word, with gender representing the strongest effect on AoA and familiarity rating, while age was the variable most influencing typicality rating. However, in terms of the number of words affected by demographic variables it seems that AoA is the lexical parameter most vulnerable to the effect of these variables as a whole (269 words out of 357 for AoA; 111 out of 405 for typicality and 121 out of 418 for familiarity).

Analysis of data across the three lexical parameters revealed no significant effects of demographic variables for typicality and familiarity, and only age significantly affected AoA ratings. More precisely, it seems that people from the 20 to 40 age range report having learnt the meaning of the words at a much younger age than people belonging to the 51-60 age range who in turn provided the highest scores across the age categories. Further investigation has to be done to clarify this pattern in the rating data.

Compared to the other lexical databases used in the literature mainly based on the rating collected on a small number of Psychology students, (Sereno, 2009) (Gilhooly, 1980) or biased for age or gender (Marques, et al., 2007) this is the only study to provide data from a large sample evenly distributed for age and gender, with a wide age range including very young adult (18 years old) and very old elderly (92 years old). Because of its characteristics, this database could therefore offer a more valid psychometric instrument in clinical and research environments. This is important especially when attention and resources are focused on a heterogeneous disorder like Alzheimer’s disease, of which the probability of detection at the preclinical stage is mainly due to the efficacy of the tools which have to be sensitive to minor cognitive changes and able to differentiate, amongst the people with mild cognitive impairment, those who have the highest chance of developing dementia. Further research is necessary to provide a validation of these rating in a clinical population.

CHAPTER 7 General discussion

To date no studies have attempted to combine data across methodologies to predict cognitive decline in conjunction with genetic status. The focus of these experiments were to build up useful endophenotypes to better understand the nature of genetic late-onset AD associated with structural and functional brain changes and cognitive reserve mechanisms acting as prevention to further cognitive impairment. In particular the aims of this dissertation were to explore whether the presence of the ApoE ε4 allele in individuals with mild cognitive impairment is associated with a pattern of more severe lexical-semantic deficit as observed in AD patients (Forbes-McKay, et al., 2005). A further aim of this dissertation was to investigate if there was any interaction between ApoE ε4 related structural loss in areas of the brain associated with verbal fluency decline and the presence of lexical effects in individuals at the preclinical stage of AD and thus to identify which brain areas are responsible for the preclinical lexical effects observed in MCI patients. A final aim was to generate a set of age, gender and education based norms for those lexical characteristics of the words (AoA, typicality and familiarity) which previous study in this field have focused on, in order to build up valid psychometric parameters able to detect subtle lexical deficits in healthy people carriers of the ApoE ε4 allele.

7.1 ApoE ε4 related Cognitive impairments

In line with other findings Forbes-McKay, et al., 2005; Holmes, Fitch, et al., 2006) the AD group, when tested with a semantic fluency task, performed significantly differently from controls producing a smaller number of words. There were also significant differences in the mean lexical characteristic values (see Chapter 4, section 4.2.5). In particular, patients produced fewer words and those were more imageable, more typical of their category and acquired earlier than those produced by healthy elderly individuals. When these results were analysed according to the presence of the ApoE ε4 across the groups no significant lexical differences were found between AD carriers/AD non-carriers and controls. However, the mean mean typicality values produced by AD carriers were higher than those of controls and the significance level was higher than that observed between AD non-carriers and controls ($p < .001$ and $p < .05$ respectively), although no significant differences were found when the two genetically different subgroups were directly compared. In AD, the effect of genotype was strong overall but only partially detectable on the semantic indices. Several factors may account for the non-significant difference between carriers and non-carriers. One factor has to do with the age range of the participants examined in this study. Some studies have reported that the risk of AD development associated with ε4 status appears to peak by the age of 70 and to dissipate up to the age of 80 (Blacker, Haines, Rodes, Terwedow, Go, et al., 1997; Breitner, et al., 1999). The AD carriers' mean age was 77.22 years; this high mean age might have weakened the influence of the ε4 allele. This emphasises the need to consider age as a variable in the analyses when the effect of the ApoE is taken into account on potential cognitive phenotypes. Another possible factor that might have affected the study concerns a dose-response relationship in exhibiting ε4-related deficits, where ε4 homozygotes perform the worst and non-ε4carriers perform the best (Caselli, et al., 2007; L. G. Nilsson, et al., 2006). In the study, homozygosity for ε4 was not separated from the heterozygosity on ε4 because of the small sample number for each subgroup, possibly further diluting the behavioral outcome at this already advanced stage of cognitive deterioration. Finally, the sample size could have been another key factor with only 19 AD ApoE ε4 carriers (8 ε4ε4) and 10 non-carriers compared to other studies such as that of Forbes-Mckay et al. (2005) who tested 96 AD.

To see if it would have been easier to detect subtle semantic deficits in the preclinical stage of AD, the same investigation was replicated in a small sample of amnestic MCI carriers and non-carriers of the ApoE ε4 allele. A clearer picture could be seen from these results although still no significant differences were found. However, the results showed that the presence of at least one ε4 (there were no homozygous carriers of the ε4 allele in the MCI sample) influenced the way in which patients carrying this allele produce animal and fruit words. E4 non-carriers performed better and their pattern was the most different from that of the carriers (typicality and familiarity: ε4 carriers > controls > ε4 non-carriers; age of acquisition: ε4 carriers < controls < ε4 non-carriers).

When the hypothesis of poorer lexical skills retrieval in MCI carriers was tested in a bigger sample of amnestic MCI individuals (18 aMCI carriers and 12 non-carriers) it was finally found that a more severe impoverishment of spontaneous language for the aMCI carriers was characterised by significant lexical effects. The words generated by aMCI ε4 carriers were earlier acquired, more familiar, and more typical of their semantic category than the words generated by healthy controls. Interestingly, age of acquisition values were the only values, among all the other lexical features examined, to differentiate the performance of carriers and non-carriers from controls. These findings corroborate the results from Forbes-Mckay (2005) which showed that AoA was the best predictor of group membership (patient/control). It seems, therefore, that age of acquisition is the lexical parameter most sensitive to the earliest pathological changes due to AD which can be detected at a preclinical stage when the neuropathological impairment is minimal.

Examination of group differences revealed that aMCI carriers scored significantly lower on the category fluency task than aMCI non-carriers. There was no significant difference, however, on the phonemic fluency test. This result confirms earlier findings which have shown that MCI patients experienced difficulties in making semantic associations between exemplars of subcategories rather than having more general difficulties searching through lexical representations (Murphy, et al., 2006; Saxton, et al., 2004). Moreover our results suggest that there is deterioration of brain areas beyond that of the hippocampus (i.e. areas supporting semantic memory) at this early stage. Similar results have been found by Venneri and colleagues (Venneri, et al., 2008) with mild AD. These authors found strong lexical effects (typicality and age of acquisition) in patients showing grey matter loss in perirhinal and parahippocampal cortex. It seems, therefore, that performance on semantic fluency task relies more on temporal than frontal structures. In particular, the temporal lobe seems to be used for both tasks (semantic and phonemic), while the frontal lobe appears to provide its major contribution only to letter fluency (Julie D. Henry, Crawford, & Phillips, 2004). It could be that during the life course the ApoE ε4 allele interacts with AD pathology affecting some areas more sensitive to its early effect and involved in the retrieval from long term semantic memory (such as mediotemporal structures). This hypothesis is supported by other studies which have shown lower metabolism in areas associated with lexical semantic representations in asymptomatic carriers of the ApoE ε4 allele [e.g (Reiman, et al., 2004)].

7.2 ApoE ε4 related neuroanatomical substrates during semantic memory task.

The behavioural findings indicated that subtle lexical deficits in aMCI ε4 carriers could have a role as possible cognitive endophenotype of AD. It remains to be tested whether this cognitive endophenotype was associated with an ApoE ε4 related pattern of atrophy in regions supporting semantic retrieval from long term memory in aMCI and mild to minimal AD carriers. Behavioural results showed significant impoverishment of lexical semantic abilities in general, with the MCI ε4 carriers showing the worst cognitive output of all, although this was only detectable as a trend. Volumetric analyses identified differences in the pattern of grey matter volume loss among the three groups according to the presence of the ApoE ε4 mutation, and the degree of semantic retrieval competency measured through the mean lexical characteristic values produced by each person belonged to each group. In the aMCI subgroups there was a pattern characterised by atrophy confined to mediotemporal areas and necortical regions bilaterally with greater involvement of mediotemporal structures, the posterior cingulate and parietal cortex. Non-carriers had more widespread atrophy. These grey matter volume differences within the two aMCI groups also became more evident in the direct aMCI ε4 carriers/non-carriers comparison (smaller grey matter volume in the parietal regions and in the temporal lobe for the aMCI non-carriers and more specific atrophy in the parahippocampal gyrus for the aMCI carriers). A correlation analysis was also run which showed an association between poorer retrieval skills and atrophy in the left parietal, medial temporal and frontal regions and cerebellum bilaterally. Finally, a conjunction analysis was carried out to identify regions of significant overlap between volume differences in aMCI carrier vs non-carriers healthy controls and areas of significant correlation of grey matter volume and AoA values. This analysis underlies the role of the parahippocampal gyrus, caudate nucleus and thalamus as areas associated with retrieval of later acquired words in the category fluency task when associated to the ApoE ε4. Several conclusions can be drawn from these results. Firstly, in line with the few other neuroimaging studies investigating brain morphology in MCI carriers and non-carriers (Morra, et al., 2009; Thomann, et al., 2008; Pennanen, et al., 2006), our morphometric results showed the significant impact of the ApoE ε4 burden on the brain atrophy of people at the preclinical stage of AD. Atrophy patterns that we also found in the study comparing grey matter volume in AD carriers and non-carriers. It seems, therefore, that the ApoE ε4 allele plays a role in modulating the anatomical expression of the disease. In addition, this study is the first to examine the interplay between the effect of the ApoE ε4 burden on neuropathological substrates and the degraded lexical semantic skills in a sample of aMCI. If the focus is on the different degree of semantic impairment across the MCI carriers/non-carriers and controls in relation to the difference in the pattern of grey matter atrophy it seems that more restricted whole brain atrophy (in particular in the parahippocampal structures) seen in MCI ε4 carriers might cause poorer semantic retrieval skills. More widespread atrophy (neocortical and mediotemporal areas) is seen in MCI non-carriers, although despite more extensive anatomical damage still delivered a slightly better semantic performance. These findings suggest that more restricted volumetric differences (limbic areas) in the MCI ε4 subgroup have greater impact on the lexical semantic deficit that ε4 carriers showed. This hypothesis is corroborated by the findings of an fMRI study which showed abnormal functioning of limbic structures in a sample of individuals at greater risk for AD (Bassett, et al., 2006). However, there are findings from previous studies which also show an association between damage in neocortical areas and poorer semantic skills in fronto-temporal dementia and early stage AD [e.g.(Venneri, et al., 2008)], corroborating the possible involvement of more neocortical atrophy in the less severe but still present semantic deficit in MCI non-carriers when compared to controls. It might be that people carrying the ε4 mutation don't possess a fully developed neuronal network which would allow them to maintain cognitive competence when areas specific to semantic retrieval skills are compromised. MCI non-carriers, instead, could count on a more structured and efficient neuronal network which would allow them compensatory processes in maintaining cognitive performance. These assumptions are in line with Mesulam's plasticity-based theory of pathogenesis of AD (Mesulam, 2000). This author posits a role for ApoE ε4 as one of the initial factors implicated in AD development creating physiological barriers toward the manifestation of the plasticity (increasing the neuroplasticity burden), neuronal repairs and cognitive reserve (see Chapter 1, section 1.7.2). It could be that the dysfunctional role of this mutation is marked phenotypically by subtle semantic deficits in the preclinical stage when the neuropathological changes due to AD are still confined and thus allow the detection of cognitive endophenotypes useful in early diagnosis. Howerer, additional larger studies are needed to validate and corroborate these results.

7.3 Lexical semantic parameters: normative data

Based on the convergent results of our previous studies supporting the meaningfulness of subtle lexical change as a possible cognitive endophenotype for the preclinical detection of AD, our last study was oriented to build a database of lexical-semantic rating which could mark the confounding effect of demographic variables and thus be used in future research investigating the reliability of the role of linguistic deficits in the preclinical diagnosis of AD. The point was to create a clinically and experimentally useful set of psychometric instruments derived from a large population sample evenly distributed for age, gender and education. To date there are no database available which have these characteristics and have provided lexical rating for which the effect of demographic variables have been studied. This study presented an age of acquisition, typicality and familiarity database showing the mean, standard deviation and range (minimum and maximum) values for 476 items (animal and fruit words).The effect of the demographic variables of interest (gender, age and education) on the mean rating value provided by healthy individuals was also presented. This database, therefore, although it needs to be validated in future research could be very useful as a clinical and research tool for the possible detection and monitoring of subtle semantic cognitive deficits in the early stage of cognitive decline and to monitor progression over time.

7.4 Conclusion

In summary, these studies have investigated the interplay between behavioral data, morphological measures and genetic burden in a population of amnestic mild cognitive impairment and mild to moderate Alzheimer's disease. The focus was on testing the sensitivity of lexical semantic parameters as cognitive endophenotypes to detect the earliest sign of AD pathology and to better clarify the association between the early anatomical changes due to the disorder and poorer lexical-semantic skills when increased genetic risk is present. The final aim was to provide a valid and reliable set of psychometric parameters to allow the identification of subtle lexical-semantic changes at the early stage of cognitive decline. For this purpose, an AoA, typicality and familiarity database was created. Additional larger studies are needed to better understand the relationship between genetic burden, structural differences and early lexical deficits in preclinical AD. This combined interdisciplinary approach, however, seems to yield a much clearer picture of the pattern of decline connecting normal and abnormal aging and provides evidence in support of the use of lexical-semantic parameters on cognitive endophenotype of this disease to aid early and differential diagnosis between normal and abnormal decline in ageing.

BIBLIOGRAPHY

Adlam, A. L. R., Bozeat, S., Arnold, R., Watson, P., & Hodges, J. R. (2006). Semantic knowledge in mild cognitive impairment and mild Alzheimer's disease. *Cortex*, *42*(5), 675-684.

Adolfsson, R., Gottfries, C. G., Roos, B. E., & Winblad, B. (1979). Changes in the brain catecholamines in patients with dementia of Alzheimer type. *Br J Psychiatry, 135*, 216-223.

Agosta, F., Vossel, K. A., Miller, B. L., Migliaccio, R., Bonasera, S. J., Filippi, M., et al. (2009). Apolipoprotein E epsilon4 is associated with disease-specific effects on brain atrophy in Alzheimer's disease and frontotemporal dementia. *Proc Natl Acad Sci U S A, 106*(6), 2018-2022.

Ahmed, S., Arnold, R., Thompson, S. A., Graham, K. S., & Hodges, J. R. (2008). Naming of objects, faces and buildings in mild cognitive impairment. *Cortex*, *44*(6), 746-752.

Alberca, R., Salas, D., Perez-Gil, J. A., Lozano, P., & Gil-Neciga, E. (1999). Verbal fluency and Alzheimer's disease. *Neurologia, 14*(7), 344-348.

Albert, M. S., Moss, M. B., Tanzi, R., & Jones, K. (2001). Preclinical prediction of AD using neuropsychological tests. *J Int Neuropsychol Soc, 7*(5), 631-639.

Alexander, G. E., Chen, K., Pietrini, P., Rapoport, S. I., & Reiman, E. M. (2002). Longitudinal PET Evaluation of Cerebral Metabolic Decline in Dementia: A Potential Outcome Measure in Alzheimer's Disease Treatment Studies. *Am J Psychiatry, 159*(5), 738-745.

Alexander, G. E., Chen, K., & Reiman, E. M. (2003). *APOE e4 dose effect on grey matter atrophy in cognitively normal adults*. Paper presented at the *33rd Society for Neuroscience Conference Annual meeting*.

Almkvist, O., Axelman, K., Basun, H., Wahlund, L. O., & Lannfelt, L. (2002). Conversion from preclinical to dinical stage of Alzheimer's disease as shown by decline of cognitive function in carriers of the Swedish APP-mutation. *J Neural Transm Suppl*(62), 117-125.

Almkvist, O., Basum,H.,Wahuld,L.O., & Lannfelt,L. (1995). Cognitive functions of family members with and without the APP670/671 mutation. In J. A. M. K. Iqbal, B. Winbland,& H.M. Wisniewsky (Ed.), *Research advance in Alzheimer's disease and related disorder* (pp. 251-259). New York: John Wiley.

Alvarez-Arcaya, A., Combarros, O., Llorca, J., Sanchez-Guerra, M., Berciano, J., & Fernandez-Luna, J. L. (2001). The 491 TT apolipoprotein E promoter polymorphism is associated with reduced risk for sporadic Alzheimer's disease. *Neurosci Lett, 304*(3), 204-208.

Amar, K., MacGowan, S., Wilcock, G., Lewis, T., & Scott, M. (1998). Are genetic factors important in the aetiology of leukoaraiosis? Results from a memory clinic population. *Int J Geriatr Psychiatry, 13*(9), 585-590.

Amieva, H., Le Goff, M., Millet, X., Orgogozo, J. M., Peres, K., Barberger-Gateau, P., et al. (2008). Prodromal Alzheimer's Disease: Successive Emergence of the Clinical Symptoms. *Annals of Neurology, 64*(5), 492-498.

Anchisi, D., Borroni, B., Franceschi, M., Kerrouche, N., Kalbe, E., Beuthien-Beumann, B., et al. (2005). Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer disease. *Arch Neurol*, *62*(11), 1728-1733.

Andersen, K., Launer, L. J., Dewey, M. E., Letenneur, L., Ott, A., Copeland, J. R., et al. (1999). Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence Research Group. *Neurology, 53*(9), 1992-1997.

Anstey, K., & Christensen, H. (2000). Education, activity, health, blood pressure and apolipoprotein E as predictors of cognitive change in old age: A review. *Gerontology, 46*(3), 163-177.

Appell, J., Kertesz, A., & Fisman, M. (1982). A study of language functioning in Alzheimer patients. *Brain Lang, 17*(1), 73-91.

Arango-Lasprilla, J. C., Cuetos, F., Valencia, C., Uribe, C., & Lopera, F. (2007). Cognitive changes in the preclinical phase of familial Alzheimer's disease. *J Clin Exp Neuropsychol, 29*(8), 892-900.

Arango Lasprilla, J. C., Iglesias, J., & Lopera, F. (2003). Neuropsychological study of familial Alzheimer's disease caused by mutation E280A in the presenilin 1 gene. *Am J Alzheimers Dis Other Demen, 18*(3), 137-146.

Arango-Lasprilla, J. C., Rogers, H., Lengenfelder, J., Deluca, J., Moreno, S., & Lopera, F. (2006). Cortical and subcortical diseases: Do true neuropsychological differences exist? *Arch Clin Neuropsychol, 21*(1), 29-40.

Arendt, T., Schindler, C., Bruckner, M. K., Eschrich, K., Bigl, V., Zedlick, D., et al. (1997). Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein epsilon 4 allele. *J Neurosci, 17*(2), 516-529.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., & Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb Cortex, 1*(1), 103-116.

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., & Hyman, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology, 42*(3 Pt 1), 631-639.

Ashford, J. W., & Mortimer, J. A. (2002). Non-familial Alzheimer's disease is mainly due to genetic factors. *J Alzheimers Dis, 4*(3), 169-177.

Auriacombe, S., Lechevallier, N., Amieva, H., Harston, S., Raoux, N., & Dartigues, J. F. (2006). A longitudinal study of quantitative and qualitative features of category verbal fluency in incident Alzheimer's disease subjects: Results from the PAQUID study. *Dement Geriatr Cogn Disord, 21*(4), 260-266.

Baayen, R. H. (1995). Lexical Acquisition Exploiting Online Resources to Build a Lexicon *Linguistics, 33*(2), 396-398.

Bachman, D. L., Wolf, P. A., Linn, R. T., Knoefel, J. E., Cobb, J. L., Belanger, A. J., et al. (1993). Incidence of dementia and probable Alzheimer's disease in a general population: The Framingham Study. *Neurology, 43*(3 Pt 1), 515-519.

Backman, L., Small, B. J., & Fratiglioni, L. (2001). Stability of the preclinical episodic memory deficit in Alzheimer's disease. *Brain, 124*(Pt 1), 96-102.

Bacskai, B. J., Klunk, W. E., Mathis, C. A., & Hyman, B. T. (2002). Imaging amyloid-beta deposits in vivo. *J Cereb Blood Flow Metab, 22*(9), 1035-1041.

Bagnoli, S., Nacmias, B., Tedde, A., Guarnieri, B. M., Cellini, E., Ciantelli, M., et al. (2002). Cathepsin D polymorphism in Italian sporadic and familial Alzheimer's disease. *Neurosci Lett, 328*(3), 273-276.

Baldereschi, M., Di Carlo, A., Lepore, V., Bracco, L., Maggi, S., Grigoletto, F., et al. (1998). Estrogen-replacement therapy and Alzheimer's disease in the Italian Longitudinal Study on Aging. *Neurology, 50*(4), 996-1002.

Bales, K. R., Verina, T., Cummins, D. J., Du, Y., Dodel, R. C., Saura, J., et al. (1999). Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A, 96*(26), 15233-15238.

Banati, R. B., Gehrmann, J., Czech, C., Monning, U., Jones, L. L., Konig, G., et al. (1993). Early and rapid de novo synthesis of Alzheimer beta A4-amyloid precursor protein (APP) in activated microglia. *Glia, 9*(3), 199-210.

Barbeau, E. J., Ranjeva, J. P., Didic, A., Confort-Gouny, S., Felician, O., Soulier, E., et al. (2008). Profile of memory impairment and gray matter loss in amnestic mild cognitive impairment. *Neuropsychologia, 46*(4), 1009-1019.

Barnes, J., Bartlett, J. W., van de Pol, L. A., Loy, C. T., Scahill, R. I., Frost, C., et al. (2009). A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol Aging, 30*(11), 1711-1723.

Baron, J. C., Chetelat, G., Desgranges, B., Perchey, G., Landeau, B., de la Sayette, V., et al. (2001). In vivo mapping of gray matter loss with voxel-based morphometry in mild Alzheimer's disease. *Neuroimage, 14*(2), 298-309.

Bassett, S. S., Yousem, D. M., Cristinzio, C., Kusevic, I., Yassa, M. A., Caffo, B. S., et al. (2006). Familial risk for Alzheimer's disease alters fMRI activation patterns. *Brain, 129*, 1229-1239.

Baxter, L. C., Caselli, R. J., Johnson, S. C., Reiman, E., & Osborne, D. (2003). Apolipoprotein E epsilon 4 affects new learning in cognitively normal individuals at risk for Alzheimer's disease. *Neurobiol Aging, 24*(7), 947-952.

Bayles, K. A., Kaszniak, A. W., & Tomoeda, C. K. (1987). *Communication and cognition in normal aging and dementia.* Boston, MA,USA: College-Hill Press.

Bayles, K. A., & Tomoeda, C. K. (1983). Confrontation naming impairment in dementia. *Brain Lang, 19*(1), 98-114.

Becher, J. C., Keeling, J. W., McIntosh, N., Wyatt, B., & Bell, J. (2006). The distribution of apolipoprotein E alleles in Scottish perinatal deaths. *J Med Genet, 43*(5), 414-418.

Becker, J. T., Mintun, M. A., Aleva, K., Wiseman, M. B., Nichols, T., & DeKosky, S. T. (1996). Compensatory reallocation of brain resources supporting verbal episodic memory in Alzheimer's disease. *Neurology, 46*(3), 692-700.

Beeson, J. G., Shelton, E. R., Chan, H. W., & Gage, F. H. (1994). Age and damage induced changes in amyloid protein precursor immunohistochemistry in the rat brain. *J Comp Neurol, 342*(1), 69-77.

Beffert, U., & Poirier, J. (1996). Apolipoprotein E, plaques, tangles and cholinergic dysfunction in Alzheimer's disease. *Ann N Y Acad Sci, 777*, 166-174.

Bell-McGinty, S., Lopez, O. L., Meltzer, C. C., Scanlon, J. M., Whyte, E. M., Dekosky, S. T., et al. (2005). Differential cortical atrophy in subgroups of mild cognitive impairment. *Arch Neurol, 62*(9), 1393-1397.

Bellosta, S., Nathan, B. P., Orth, M., Dong, L. M., Mahley, R. W., & Pitas, R. E. (1995). Stable expression and secretion of apolipoproteins E3 and E4 in mouse neuroblastoma cells produces differential effects on neurite outgrowth. *J Biol Chem, 270*(45), 27063-27071.

Bennett, D. A., Schneider, J. A., Wilson, R. S., Bienias, J. L., & Arnold, S. E. (2004). Neurofibrillary tangles mediate the association of amyloid load with clinical

Alzheimer disease and level of cognitive function. *Arch Neurol, 61*(3), 378-384.

Bennett, D. A., Schneider, J. A., Wilson, R. S., Bienias, J. L., Berry-Kravis, E., & Arnold, S. E. (2005). Amyloid mediates the association of apolipoprotein E e4 allele to cognitive function in older people. *J Neurol Neurosurg Psychiatry, 76*(9), 1194-1199.

Bennett, D. A., Wilson, R. S., Schneider, J. A., Evans, D. A., Beckett, L. A., Aggarwal, N. T., et al. (2002). Natural history of mild cognitive impairment in older persons. *Neurology, 59*(2), 198-205.

Berg, L., McKeel, D. W., Jr., Miller, J. P., Storandt, M., Rubin, E. H., Morris, J. C., et al. (1998). Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease: Relation of histologic markers to dementia severity, age, sex, and apolipoprotein E genotype. *Arch Neurol, 55*(3), 326-335.

Bergem, A. L., Engedal, K., & Kringlen, E. (1997). The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. *Arch Gen Psychiatry, 54*(3), 264-270.

Bergman, Y., & Mostoslavsky, R. (1998). DNA demethylation: Turning genes on. *Biol Chem, 379*(4-5), 401-407.

Berlingeri, M., Bottini, G., Basilico, S., Silani, G., Zanardi, G., Sberna, M., et al. (2006, Oct). *Anatomy of the episodic buffer: A voxel-based morphometry study in patients with dementia.* Paper presented at the 2nd Meeting of the European Society of Neuropsychology, Toulouse, FRANCE.

Bertram, C. G., Gaut, R. M., Barrett, J. H., Pinney, E., Whitaker, L., Turner, F., et al. (2002). An assessment of the CDKN2A variant Ala148Thr as a nevus/melanoma susceptibility allele. *J Invest Dermatol, 119*(4), 961-965.

Bertram, L. (2009). Alzheimer's disease genetic current status and future perspectives. *Int Rev Neurobiol, 84*, 167-184.

Bertram, L., Blacker, D., Crystal, A., Mullin, K., Keeney, D., Jones, J., et al. (2000). Candidate genes showing no evidence for association or linkage with Alzheimer's disease using family-based methodologies. *Exp Gerontol, 35*(9-10), 1353-1361.

Bertram, L., Hsiao, M., McQueen, M. B., Parkinson, M., Mullin, K., Blacker, D., et al. (2007). The LDLR locus in Alzheimer's disease: A family-based study and meta-analysis of case-control data. *Neurobiol Aging, 28*(1), 11-14.

Bertram, L., Lange, C., Mullin, K., Parkinson, M., Hsiao, M., Hogan, M. F., et al. (2008a). Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet, 83*(5), 623-632.

Bertram, L., McQueen, M. B., Mullin, K., Blacker, D., & Tanzi, R. E. (2007). Systematic meta-analyses of Alzheimer disease genetic association studies: The AlzGene database. *Nat Genet, 39*(1), 17-23.

Binetti, G., Signorini, S., Squitti, R., Alberici, A., Benussi, L., Cassetta, E., et al. (2003). Atypical dementia associated with a novel presenilin-2 mutation. *Ann Neurol, 54*(6), 832-836.

Bird, H., Ralph, M. A. L., Patterson, K., & Hodges, J. R. (2000). The rise and fall of frequency and imageability: Noun and verb production in semantic dementia. *Brain and Language, 73*(1), 17-49.

Birn, R. M., Kenworthy, L., Case, L., Caravella, R., Jones, T. B., Bandettini, P. A., et al. Neural systems supporting lexical search guided by letter and semantic category cues: A self-paced overt response fMRI study of verbal fluency. *Neuroimage, 49*(1), 1099-1107.

Bizzozero, I., Costato, D., Sala, S. D., Papagno, C., Spinnler, H., & Venneri, A. (2000). Upper and lower face apraxia: Role of the right hemisphere. *Brain, 123 (Pt 11)*, 2213-2230.

Blacker, D., Haines, J. L., Rodes, L., Terwedow, H., Go, R. C., Harrell, L. E., et al. (1997). ApoE-4 and age at onset of Alzheimer's disease: The NIMH genetics initiative. *Neurology, 48*(1), 139-147.

Blackwell, A. D., Sahakian, B. J., Vesey, R., Semple, J. M., Robbins, T. W., & Hodges, J. R. (2004). Detecting dementia: Novel neuropsychological markers of preclinical Alzheimer's disease. *Dement Geriatr Cogn Disord, 17*(1-2), 42-48.

Blangero, J., Williams, J. T., & Almasy, L. (2003). Novel family-based approaches to genetic risk in thrombosis. *J Thromb Haemost, 1*(7), 1391-1397.

Blennow, K., de Leon, M. J., & Zetterberg, H. (2006). Alzheimer's disease. *Lancet, 368*(9533), 387-403.

Boccardi, M., Sabattoli, F., Testa, C., Beltramello, A., Soininen, H., & Frisoni, G. B. (2004). APOE and modulation of Alzheimer's and frontotemporal dementia. *Neurosci Lett, 356*(3), 167-170.

Boly, M., Phillips, C., Tshibanda, L., Vanhaudenhuyse, A., Schabus, M., Dang-Vu, T. T., et al. (2008). Intrinsic brain activity in altered states of consciousness: How conscious is the default mode of brain function? *Ann N Y Acad Sci, 1129*, 119-129.

Bondi, M. W., Houston, W. S., Eyler, L. T., & Brown, G. G. (2005). fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology, 64*(3), 501-508.

Bondi, M. W., Houston, W. S., Salmon, D. P., Corey-Bloom, J., Katzman, R., Thal, L. J., et al. (2003). Neuropsychological deficits associated with Alzheimer's disease in the very-old: Discrepancies in raw vs. standardized scores. *J Int Neuropsychol Soc, 9*(5), 783-795.

Bondi, M. W., Salmon, D. P., Galasko, D., Thomas, R. G., & Thal, L. J. (1999). Neuropsychological function and apolipoprotein E genotype in the preclinical detection of Alzheimer's disease. *Psychol Aging, 14*(2), 295-303.

Bondi, M. W., Salmon, D. P., Monsch, A. U., Galasko, D., Butters, N., Klauber, M. R., et al. (1995). Episodic memory changes are associated with the APOE-epsilon 4 allele in nondemented older adults. *Neurology, 45*(12), 2203-2206.

Bondi, M. W., Serody, A. B., Chan, A. S., Eberson-Shumate, S. C., Delis, D. C., Hansen, L. A., et al. (2002). Cognitive and neuropathologic correlates of Stroop Color-Word Test performance in Alzheimer's disease. *Neuropsychology, 16*(3), 335-343.

Bookheimer, S., & Burggren, A. (2009). APOE-4 genotype and neurophysiological vulnerability to Alzheimer's and cognitive aging. *Annu Rev Clin Psychol, 5*, 343-362.

Bookheimer, S. Y., Strojwas, M. H., Cohen, M. S., Saunders, A. M., Pericak-Vance, M. A., Mazziotta, J. C., et al. (2000). Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med, 343*(7), 450-456.

Bowen, D. M., Allen, S. J., Benton, J. S., Goodhardt, M. J., Haan, E. A., Palmer, A. M., et al. (1983). Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. *J Neurochem, 41*(1), 266-272.

Bowirrat, A., Treves, T. A., Friedland, R. P., & Korczyn, A. D. (2001). Prevalence of Alzheimer's type dementia in an elderly Arab population. *Eur J Neurol, 8*(2), 119-123.

Boyle, P. A., Buchman, A. S., Wilson, R. S., Kelly, J. F., & Bennett, D. A. (2010). The APOE epsilon 4 Allele Is Associated with Incident Mild Cognitive Impairment among Community-Dwelling Older Persons. *Neuroepidemiology, 34*(1), 43-49.

Bozzali, M., Filippi, M., Magnani, G., Cercignani, M., Franceschi, M., Schiatti, E., et al. (2006a). The contribution of voxel-based morphometry in staging patients with mild cognitive impairment. *Neurology, 67*(3), 453-460.

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

Braak, H., & Braak, E. (1996). Evolution of the neuropathology of Alzheimer's disease. *Acta Neurol Scand Suppl, 165*, 3-12.

Brecht, W. J., Harris, F. M., Chang, S., Tesseur, I., Yu, G. Q., Xu, Q., et al. (2004). Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. *J Neurosci, 24*(10), 2527-2534.

Breitner, J. C., Wyse, B. W., Anthony, J. C., Welsh-Bohmer, K. A., Steffens, D. C., Norton, M. C., et al. (1999). APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: The Cache County Study. *Neurology, 53*(2), 321-331.

Breslow, N. E., & Day, N. E. (1987). Statistical methods in cancer research. Volume II--The design and analysis of cohort studies. *IARC Sci Publ*(82), 1-406.

Breteler, M. M. (2000). Vascular risk factors for Alzheimer's disease: An epidemiologic perspective. *Neurobiol Aging, 21*(2), 153-160.

Brickell, K. L., Leverenz, J. B., Steinbart, E. J., Rumbaugh, M., Schellenberg, G. D., Nochlin, D., et al. (2007). Clinicopathological concordance and discordance in three monozygotic twin pairs with familial Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 78*(10), 1050-1055.

Brion, J. P., Octave, J. N., & Couck, A. M. (1994). Distribution of the phosphorylated microtubule-associated protein tau in developing cortical neurons. *Neuroscience, 63*(3), 895-909.

Bronge, L., Fernaeus, S. E., Blomberg, M., Ingelson, M., Lannfelt, L., Isberg, B., et al. (1999). White matter lesions in Alzheimer patients are influenced by apolipoprotein E genotype. *Dement Geriatr Cogn Disord, 10*(2), 89-96.

Brscic, E., Bergerone, S., Gagnor, A., Colajanni, E., Matullo, G., Scaglione, L., et al. (2000). Acute myocardial infarction in young adults: Prognostic role of angiotensin-converting enzyme, angiotensin II type I receptor, apolipoprotein E, endothelial constitutive nitric oxide synthase, and glycoprotein IIIa genetic polymorphisms at medium-term follow-up. *Am Heart J, 139*(6), 979-984.

Brun, A., Englund, B., Gustafson, L., Passant, V., Mann, D. M. A., & Neary, D. (1994). Clinical and Neuropathological criteris for frontotemporal dementia. *Journal of Neurology, Neurosurgery, and Psychiatry., 57*, 416-418.

Bryan, J., Luszcz, M. A., & Crawford, J. R. (1997). Verbal knowledge and speed of information processing as mediators of age differences in verbal fluency performance among older adults. *Psychol Aging, 12*(3), 473-478.

Burggren, A. C., Small, G. W., Sabb, F. W., & Bookheimer, S. Y. (2002). Specificity of brain activation patterns in people at genetic risk for Alzheimer disease. *Am J Geriatr Psychiatry, 10*(1), 44-51.

Burggren, A. C., Zeineh, M. M., Ekstrom, A. D., Braskie, M. N., Thompson, P. M., Small, G. W., et al. (2008). Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E e4 carriers. *Neuroimage, 41*(4), 1177-1183.

Burns, A., & Iliffe, S. (2009). Alzheimer's disease. *BMJ, 338*, 158.

Burt, T. D., Agan, B. K., Marconi, V. C., He, W., Kulkarni, H., Mold, J. E., et al. (2008). Apolipoprotein (apo) E4 enhances HIV-1 cell entry in vitro, and the APOE epsilon4/epsilon4 genotype accelerates HIV disease progression. *Proc Natl Acad Sci U S A, 105*(25), 8718-8723.

Butters, N., Granholm, E., Salmon, D. P., Grant, I., & Wolfe, J. (1987). Episodic and semantic memory: a comparison of amnesic and demented patients. *J Clin Exp Neuropsychol, 9*(5), 479-497.

Buttini, M., Orth, M., Bellosta, S., Akeefe, H., Pitas, R. E., Wyss-Coray, T., et al. (1999). Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: Isoform-specific effects on neurodegeneration. *J Neurosci, 19*(12), 4867-4880.

Cacabelos, R. (1996). Diagnosis of Alzheimer's disease: Defining genetic profiles (genotype vs phenotype). *Acta Neurol Scand Suppl, 165*, 72-84.

Cacabelos, R. (1999a). *Handbook of Neurogeriatrics. Alzheimer's disease and Other Dementias. Epidemiology and Geretics.* Barcelona, Spain.: Masson.

Cacabelos, R. (2001). Psychogeriatric research: A conceptual introduction to geriatric neuroscience. *Psychogeriatrics, 1*, 158-188.

Cacabelos, R. (2002a). Pharmacogenomics in Alzheimer's disease. *Min. Rev. Med Chem., 2*, 59-84.

Cacabelos, R. (2002b). Pharmacogenomics for the treatment of dementia. *Annals of Medicine, 34*(5), 357-379.

Cacabelos, R., Alvarez, A., Fenandez-Novoa, L., & Lombardi, V. R. (2000). A pharmacogenomic approach to Alzheimer's disease. *Acta Neurol Scand Suppl, 176*, 12-19.

Cacabelos, R., Fernandez-Novoa, L., Lombardi, V., & Takeda, M. (2003a). Genetic variation and pharmacogenomics in Alzheimer disease. *Seishin Shinkeigaku Zasshi, 105*(1), 47-67.

Cacabelos, R., Mesa, M. D., & Fernandez-Nova, L. (1999). Association of genetic risk factors in Alzheimer's disease. *Ann. Psychiatry, 7,* 35-45.

Carmelli, D., Swan, G. E., DeCarli, C., & Reed, T. (2002). Quantitative genetic modeling of regional brain volumes and cognitive performance in older male twins. *Biol Psychol, 61*(1-2), 139-155.

Carrasquillo, M. M., Zou, F. G., Pankratz, V. S., Wilcox, S. L., Ma, L., Walker, L. P., et al. (2009). Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. *Nature Genetics, 41*(2), 192-198.

Caselli, R. J., Dueck, A. C., Osborne, D., Sabbagh, M. N., Connor, D. J., Ahern, G. L., et al. (2009). Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. *N Engl J Med, 361*(3), 255-263.

Caselli, R. J., Osborne, D., Reiman, E. M., Hentz, J. G., Barbieri, C. J., Saunders, A. M., et al. (2001). Preclinical cognitive decline in late middle-aged asymptomatic apolipoprotein E-e4/4 homozygotes: A replication study. *J Neurol Sci, 189*(1-2), 93-98.

Caselli, R. J., Reiman, E. M., Locke, D. E. C., Hutton, M. L., Hentz, J. G., Hoffman-Snyder, C., et al. (2007). Cognitive domain decline in healthy apolipoprotein E epsilon 4 Homozygotes before the diagnosis of mild cognitive impairment. *Archives of Neurology, 64*(9), 1306-1311.

Celsis, P. (2000). Age-related cognitive decline, mild cognitive impairment or preclinical Alzheimer's disease? *Ann Med, 32*(1), 6-14

Chalmers, K., Wilcock, G. K., & Love, S. (2003). APOE epsilon 4 influences the pathological phenotype of Alzheimer's disease by favouring cerebrovascular over parenchymal accumulation of A beta protein. *Neuropathol Appl Neurobiol, 29*(3), 231-238.

Chan, A. S., Butters, N., Salmon, D. P., & McGuire, K. A. (1993). Dimensionality and clustering in the semantic network of patients with Alzheimer's disease. *Psychol Aging, 8*(3), 411-419.

Chandra, V., Pandav, R., Dodge, H. H., Johnston, J. M., Belle, S. H., DeKosky, S. T., et al. (2001). Incidence of Alzheimer's disease in a rural community in India: The Indo-US study. *Neurology, 57*(6), 985-989.

Chang, C. Y., & Silverman, D. H. (2004). Accuracy of early diagnosis and its impact on the management and course of Alzheimer's disease. *Expert Rev Mol Diagn, 4*(1), 63-69.

Chang, S., ran Ma, T., Miranda, R. D., Balestra, M. E., Mahley, R. W., & Huang, Y. (2005). Lipid- and receptor-binding regions of apolipoprotein E4 fragments act in concert to cause mitochondrial dysfunction and neurotoxicity. *Proc Natl Acad Sci U S A, 102*(51), 18694-18699.

Chauvet, N., Apert, C., Dumoulin, A., Epelbaum, J., & Alonso, G. (1997). Mab22C11 antibody to amyloid precursor protein recognizes a protein associated with specific astroglial cells of the rat central nervous system characterized by their capacity to support axonal outgrowth. *J Comp Neurol, 377*(4), 550-564.

Chen, K. W., Reiman, E. M., Alexander, G. E., Caselli, R. J., Gerkin, R., Bandy, D., et al. (2007). Correlations between apolipoprotein E epsilon 4 gene dose and whole brain atrophy rates. *American Journal of Psychiatry, 164*(6), 916-921.

Chen, P., Ratcliff, G., Belle, S. H., Cauley, J. A., DeKosky, S. T., & Ganguli, M. (2000). Cognitive tests that best discriminate between presymptomatic AD and those who remain nondemented. *Neurology, 55*(12), 1847-1853.

Chen, P., Ratcliff, G., Belle, S. H., Cauley, J. A., DeKosky, S. T., & Ganguli, M. (2001). Patterns of cognitive decline in presymptomatic Alzheimer disease: A prospective community study. *Arch Gen Psychiatry, 58*(9), 853-858.

Chertkow, H., & Bub, D. (1990). Semantic Memory Loss in Dementia of Alzheimers Type What Do Various Measures Measure. *Brain, 113*, 397-417.

Chetelat, G., Desgranges, B., de la Sayette, V., Viader, F., Eustache, F., & Baron, J. C. (2003). Mild cognitive impairment: Can FDG-PET predict who is to rapidly convert to Alzheimer's disease? *Neurology, 60*(8), 1374-1377.

Chetelat, G., Landeau, B., Eustache, F., Mezenge, F., Viader, F., de la Sayette, V., et al. (2005). Using voxel-based morphometry to map the structural changes associated with rapid conversion in MCI: A longitudinal MRI study. *Neuroimage, 27*(4), 934-946.

Chiang, P. K., Gordon, R. K., Tal, J., Zeng, G. C., Doctor, B. P., Pardhasaradhi, K., et al. (1996). S-Adenosylmethionine and methylation. *Faseb J, 10*(4), 471-480.

Choi, S. W., & Friso, S. (2005). Interactions between folate and aging for carcinogenesis. *Clin Chem Lab Med, 43*(10), 1151-1157.

Chui, H. C., Tierney, M., Zarow, C., Lewis, A., Sobel, E., & Perlmutter, L. S. (1993). Neuropathologic diagnosis of Alzheimer disease: Interrater reliability in the assessment of senile plaques and neurofibrillary tangles. *Alzheimer Dis Assoc Disord, 7*(1), 48-54.

Chun, M. M., & Phelps, E. A. (1999). Memory deficits for implicit contextual information in amnesic subjects with hippocampal damage. *Nat Neurosci, 2*(9), 844-847.

Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., et al. (1992). Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature, 360*(6405), 672-674.

Clark, L. J., Gatz, M., Zheng, L., Chen, Y. L., McCleary, C., & Mack, W. J. (2009). Longitudinal verbal fluency in normal aging, preclinical, and prevalent Alzheimer's disease. *Am J Alzheimers Dis Other Demen, 24*(6), 461-468

Codemo, A., Corti, M. C., Mazzetto, G., Varotto, S., Cortella, I., Crepaldi, G., et al. (2000). Education, ApoE status and cognitive impairment in elderly: An epidemiological study in a rural setting. *Neurobiol Aging, 21*(Supplement 1), 246-246.

Cole, G. M., & Ard, M. D. (2000). Influence of lipoproteins on microglial degradation of Alzheimer's amyloid beta-protein. *Microsc Res Tech, 50*(4), 316-324.

Colton, C. A., Needham, L. K., Brown, C., Cook, D., Rasheed, K., Burke, J. R., et al. (2004). APOE genotype-specific differences in human and mouse macrophage nitric oxide production. *J Neuroimmunol, 147*(1-2), 62-67.

Convit, A., de Asis, J., de Leon, M. J., Tarshish, C. Y., De Santi, S., & Rusinek, H. (2000). Atrophy of the medial occipitotemporal, inferior, and middle temporal gyri in non-demented elderly predict decline to Alzheimer's disease. *Neurobiol Aging, 21*(1), 19-26.

Corbo, R. M., & Scacchi, R. (1999). Apolipoprotein E (APOE) allele distribution in the world: Is APOE*4 a 'thrifty' allele? *Ann Hum Genet, 63*(Pt 4), 301-310.

Corder, E. H., Jelic, V., Basun, H., Lannfelt, L., Valind, S., Winblad, B., et al. (1997). No difference in cerebral glucose metabolism in patients with Alzheimer disease and differing apolipoprotein E genotypes. *Arch Neurol, 54*(3), 273-277.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., et al. (1993). Gene Dose of Apolipoprotein-E Type-4 Allele and the Risk of Alzheimers-Disease in Late-Onset Families. *Science, 261*(5123), 921-923.

Cosentino, S., Scarmeas, N., Helzner, E., Glymour, M. M., Brandt, J., Albert, M., et al. (2008). APOE epsilon 4 allele predicts faster cognitive decline in mild Alzheimer disease. *Neurology, 70*(19 Pt 2), 1842-1849.

Costafreda, S. G., Fu, C. H., Lee, L., Everitt, B., Brammer, M. J., & David, A. S. (2006). A systematic review and quantitative appraisal of fMRI studies of verbal fluency:Role of the left inferior frontal gyrus. *Hum Brain Mapp, 27*(10), 799-810.

Courchesne, E., Chisum, H. J., Townsend, J., Cowles, A., Covington, J., Egaas, B., et al. (2000). Normal brain development and aging: Quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology, 216*(3), 672-682.

Crawford, F. C., Freeman, M. J., Schinka, J., Abdullah, L. I., Richards, D., Sevush, S., et al. (2000). The genetic association between Cathepsin D and Alzheimer's disease. *Neurosci Lett, 289*(1), 61-65.

Crivello, F., Lemaitre, H., Dufouil, C., Grassiot, B., Delcroix, N., Tzourio-Mazoyer, N., et al. (2010). Effects of ApoE-varepsilon4 allele load and age on the rates of grey matter and hippocampal volumes loss in a longitudinal cohort of 1186 healthy elderly persons. *Neuroimage, Article in press, corrected proof*.

Croisile, B., Ska, B., Brabant, M. J., Duchene, A., Lepage, Y., Aimard, G., et al. (1996). Comparative study of oral and written picture description in patients with Alzheimer's disease. *Brain Lang, 53*(1), 1-19.

Cross, S. H., Meehan, R. R., Nan, X., & Bird, A. (1997). A component of the transcriptional repressor MeCP1 shares a motif with DNA methyltransferase and HRX proteins. *Nat Genet, 16*(3), 256-259.

Csernansky, J. G., Wang, L., Swank, J., Miller, J. P., Gado, M., McKeel, D., et al. (2005). Preclinical detection of Alzheimer's disease: Hippocampal shape and volume predict dementia onset in the elderly. *Neuroimage, 25*(3), 783-792.

Cuetos, F., Aguado, G., Izura, C., & Ellis, A. W. (2002). Aphasic naming in Spanish: predictors and errors. *Brain Lang, 82*(3), 344-365.

Cuetos, F., Arango-Lasprilla, J. C., Uribe, C., Valencia, C., & Lopera, F. (2007). Linguistic changes in verbal expression: A preclinical marker of Alzheimer's disease. *J Int Neuropsychol Soc, 13*(3), 433-439.

Cuetos, F., Rosci, C., Laiacona, M., & Capitani, E. (2008). Different variables predict anomia in different subjects: a longitudinal study of two Alzheimer's patients. *Neuropsychologia, 46*(1), 249-260.

Darreh-Shori, T., Brimijoin, S., Kadir, A., Almkvist, O., & Nordberg, A. (2006). Differential CSF butyrylcholinesterase levels in Alzheimer's disease patients with the ApoE epsilon4 allele in relation to cognitive function and cerebral glucose metabolism. *Neurobiol Dis, 24*(2), 326-333.

Darvesh, S., & Hopkins, D. A. (2003). Differential distribution of butyrylcholinesterase and acetylcholinesterase in the human thalamus. *J Comp Neurol, 463*(1), 25-43.

Davatzikos, C., Fan, Y., Wu, X., Shen, D., & Resnick, S. M. (2008). Detection of prodromal Alzheimer's disease via pattern classification of magnetic resonance imaging. *Neurobiol Aging, 29*(4), 514-523.

Davies, P., & Maloney, A. J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet, 2*(8000), 1403.

de Leon, M. J., Convit, A., Wolf, O. T., Tarshish, C. Y., DeSanti, S., Rusinek, H., et al. (2001). Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/poitron-emission tomography (FDG/PET). *Proc Natl Acad Sci U S A, 98*(19), 10966-10971.

De Santi, S., de Leon, M. J., Rusinek, H., Convit, A., Tarshish, C. Y., Roche, A., et al. (2001). Hippocampal formation glucose metabolism and volume losses in MCI and AD. *Neurobiol Aging, 22*(4), 529-539.

De Stropper, B. (2000). Alzheimer's disease. Closing in on gamma-secretase. *Nature, 405*, 627-629.

Deary, I. J., Pattie, A., Taylor, M. D., Whiteman, M. C., Starr, J. M., & Whalley, L. J. (2003). Smoking and cognitive change from age 11 to age 80. *J Neurol Neurosurg Psychiatry, 74*(7), 1006-1007.

Deary, I. J., Wright, A. F., Harris, S. E., Whalley, L. J., & Starr, J. M. (2004). Searching for genetic influences on normal cognitive ageing. *Trends Cogn Sci, 8*(4), 178-184.

DeKosky, S. T., Ikonomovic, M. D., Styren, S. D., Beckett, L., Wisniewski, S., Bennett, D. A., et al. (2002). Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Ann Neurol, 51*(2), 145-155.

Del Tredici, K., Rub, U., De Vos, R. A., Bohl, J. R., & Braak, H. (2002). Where does parkinson disease pathology begin in the brain? *J Neuropathol Exp Neurol, 61*(5), 413-426.

Delis, D. C., Kaplan, E., & Kramer, J.H. (2001). *Delis-Kaplan Executive Function System (D-KEFS).* San Antonio.

Delis, D. C. M. J. K., Edith; Mckee,Richard; Kramer, Joel H.; Dennis, Gettman; (1991). Alternate form of the california verbal learning test: Development and reliability *The Clinical Neurospychologist, 5,* 154 - 162

Dementia 'losing out' to cancer in funding stakes (2010). Retrieved from http://news.bbc.co.uk/1/hi/health/8493248.stm

den Heijer, T., Oudkerk, M., Launer, L. J., van Duijn, C. M., Hofman, A., & Breteler, M. M. (2002). Hippocampal, amygdalar, and global brain atrophy in different apolipoprotein E genotypes. *Neurology, 59*(5), 746-748.

Devanand, D. P., Liu, X., Tabert, M. H., Pradhaban, G., Cuasay, K., Bell, K., et al. (2008). Combining early markers strongly predicts conversion from mild cognitive impairment to Alzheimer's disease. *Biol Psychiatry, 64*(10), 871-879.

Devanand, D. P., Pelton, G. H., Zamora, D., Liu, X., Tabert, M. H., Goodkind, M., et al. (2005). Predictive utility of apolipoprotein E genotype for Alzheimer disease in outpatients with mild cognitive impairment. *Arch Neurol, 62*(6), 975-980.

Di Carlo, A., Baldereschi, M., Amaducci, L., Lepore, V., Bracco, L., Maggi, S., et al. (2002). Incidence of dementia, Alzheimer's disease, and vascular dementia in Italy. The ILSA Study. *J Am Geriatr Soc, 50*(1), 41-48.

Di Fede, G., Catania, M., Morbin, M., Rossi, G., Suardi, S., Mazzoleni, G., et al. (2009). A recessive mutation in the APP gene with dominant-negative effect on amyloidogenesis. *Science, 323*(5920), 1473-1477.

Di Paola, M., Macaluso, E., Carlesimo, G. A., Tomaiuolo, F., Worsley, K. J., Fadda, L., et al. (2007). Episodic memory impairment in patients with Alzheimer's disease is correlated with entorhinal cortex atrophy. *Journal of Neurology, 254*(6), 774-781.

Dickerson, B. C., Salat, D. H., Greve, D. N., Chua, E. F., Rand-Giovannetti, E., Rentz, D. M., et al. (2005). Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. *Neurology, 65*(3), 404-411.

Doody, R. S., Azher, S. N., Haykal, H. A., Dunn, J. K., Liao, T., & Schneider, L. (2000). Does APO epsilon4 correlate with MRI changes in Alzheimer's disease? *J Neurol Neurosurg Psychiatry, 69*(5), 668-671.

Drzezga, A., Grimmer, T., Riemenschneider, M., Lautenschlager, N., Siebner, H., Alexopoulus, P., et al. (2005). Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. *J Nucl Med, 46*(10), 1625-1632.

Drzezga, A., Lautenschlager, N., Siebner, H., Riemenschneider, M., Willoch, F., Minoshima, S., et al. (2003). Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: A PET follow-up study. *Eur J Nucl Med Mol Imaging, 30*(8), 1104-1113.

Duara, R., Barker, W. W., Lopez-Alberola, R., Loewenstein, D. A., Grau, L. B., Gilchrist, D., et al. (1996). Alzheimer's disease: Interaction of apolipoprotein E genotype, family history of dementia, gender, education, ethnicity, and age of onset. *Neurology, 46*(6), 1575-1579.

Duong, A., Whitehead, V., Hanratty, K., & Chertkow, H. (2006). The nature of lexico-semantic processing deficits in mild cognitive impairment. *Neuropsychologia, 44*(10), 1928-1935.

Durany, N., Michel, T., Kurt, J., Cruz-Sanchez, F. F., Cervos-Navarro, J., & Riederer, P. (2000). Brain-derived neurotrophic factor and neurotrophin-3 levels in Alzheimer's disease brains. *Int J Dev Neurosci, 18*(8), 807-813.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*(2), 257-269.

Elias, M. F., Beiser, A., Wolf, P. A., Au, R., White, R. F., & D'Agostino, R. B. (2000). The preclinical phase of alzheimer disease: A 22-year prospective study of the Framingham Cohort. *Arch Neurol, 57*(6), 808-813.

Elshourbagy, N. A., Liao, W. S., Mahley, R. W., & Taylor, J. M. (1985). Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc Natl Acad Sci U S A, 82*(1), 203-207.

Estevez-Gonzalez, A., Garcia-Sanchez, C., Boltes, A., Otermin, P., Pascual-Sedano, B., Gironell, A., et al. (2004). Semantic knowledge of famous people in mild cognitive impairment and progression to Alzheimer's disease. *Dement Geriatr Cogn Disord, 17*(3), 188-195.

Estevez-Gonzalez, A., Kulisevsky, J., Boltes, A., Otermin, P., & Garcia-Sanchez, C. (2003). Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: Comparison with mild cognitive impairment and normal aging. *Int J Geriatr Psychiatry, 18*(11), 1021-1028.

Etcheverria, I., Amado, L., Pichel, V., & Cacabelos, R. (2003). *Differential brain atrophy profile associated with the APOE genotype in the Spanish population*. Paper presented at the 6th International Conference on Stroke and 3th Conference of the Mediterranean Stroke Society.

.

Fagan, A. M., Holtzman, D. M., Munson, G., Mathur, T., Schneider, D., Chang, L. K., et al. (1999). Unique lipoproteins secreted by primary astrocytes from wild type, apoE (-/-), and human apoE transgenic mice. *J Biol Chem, 274*(42), 30001-30007.

Fagan, A. M., Mintun, M. A., Mach, R. H., Lee, S. Y., Dence, C. S., Shah, A. R., et al. (2006). Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. *Ann Neurol, 59*(3), 512-519.

Farlow, M., Murrell, J., Ghetti, B., Unverzagt, F., Zeldenrust, S., & Benson, M. (1994). Clinical characteristics in a kindred with early-onset Alzheimer's disease and their linkage to a G-->T change at position 2149 of the amyloid precursor protein gene. *Neurology, 44*(1), 105-111.

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *Jama, 278*(16), 1349-1356.

Farris, W., Leissring, M. A., Hemming, M. L., Chang, A. Y., & Selkoe, D. J. (2005). Alternative splicing of human insulin-degrading enzyme yields a novel isoform with a decreased ability to degrade insulin and amyloid beta-protein. *Biochemistry, 44*(17), 6513-6525.

Fassbender, K., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., et al. (2001). Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc Natl Acad Sci U S A, 98*(10), 5856-5861.

Felsenfeld, G., & Groudine, M. (2003). Controlling the double helix. *Nature, 421*(6921), 448-453.

Ferguson, S. C., Deary, I. J., Perros, P., Evans, J. C., Ellard, S., Hattersley, A. T., et al. (2003). Apolipoprotein-e influences aspects of intellectual ability in type 1 diabetes. *Diabetes, 52*(1), 145-148.

Fernaeus, S. E., Almkvist, O., Bronge, L., Ostberg, P., Hellstrom, A., Winblad, B., et al. (2001). White matter lesions impair initiation of FAS flow. *Dement Geriatr Cogn Disord, 12*(1), 52-56.

Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet, 366*(9503), 2112-2117.

Filippini, N., MacIntosh, B. J., Hough, M. G., Goodwin, G. M., Frisoni, G. B., Smith, S. M., et al. (2009a). Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A, 106*(17), 7209-7214.

Filippini, N., Rao, A., Wetten, S., Gibson, R. A., Borrie, M., Guzman, D., et al. (2009). Anatomically-distinct genetic associations of APOE epsilon 4 allele load with regional cortical atrophy in Alzheimer's disease. *Neuroimage, 44*(3), 724-728.

Fleisher, A., Grundman, M., Jack, C. R., Jr., Petersen, R. C., Taylor, C., Kim, H. T., et al. (2005). Sex, apolipoprotein E epsilon 4 status, and hippocampal volume in mild cognitive impairment. *Arch Neurol, 62*(6), 953-957.

Fleisher, A. S., Houston, W. S., Eyler, L. T., Frye, S., Jenkins, C., Thal, L. J., et al. (2005a). Identification of Alzheimer disease risk by functional magnetic resonance imaging. *Arch Neurol, 62*(12), 1881-1888.

Fleisher, A. S., Sherzai, A., Taylor, C., Langbaum, J. B., Chen, K., & Buxton, R. B. (2009). Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *Neuroimage, 47*(4), 1678-1690.

Fleisher, A. S., Sun, S., Taylor, C., Ward, C. P., Gamst, A. C., Petersen, R. C., et al. (2008). Volumetric MRI vs clinical predictors of Alzheimer disease in mild cognitive impairment. *Neurology, 70*(3), 191-199.

Folstein, M. F., Folstein, S. E., & Mchugh, P. R. (1975). Mini-Mental State Practical Method for Grading Cognitive State of Patients for Clinician. *Journal of Psychiatric Research, 12*(3), 189-198.

Fontecave, M., Atta, M., & Mulliez, E. (2004). S-adenosylmethionine: Nothing goes to waste. *Trends Biochem Sci, 29*(5), 243-249.

Forbes-McKay, K. E., Ellis, A. W., Shanks, M. F., & Venneri, A. (2005). The age of acquisition of words produced in a semantic fluency task can reliably differentiate normal from pathological age related cognitive decline. *Neuropsychologia, 43*(11), 1625-1632.

Forbes-McKay, K. E., Shanks, M. F., & Venneri, A. (2004). The evolution of dysgraphia in Alzheimer's disease. *Brain Res Bull, 63*(1), 19-24.

Forbes-McKay, K. E., & Venneri, A. (2003). A case for case: Handling letter case selection in written spelling. *Neuropsychologia, 41*(1), 16-24.

Forbes-McKay, K. E., & Venneri, A. (2005). Detecting subtle spontaneous language decline in early Alzheimer's disease with a picture description task. *Neurol Sci, 26*(4), 243-254.

Forbes-McKay, K. E., Venneri, A., & Shanks, M. F. (2002). Distinct patterns of spontaneous speech deterioration: An early predictor of Alzheimer's disease. *Brain Cogn, 48*(2-3), 356-361.

Forbes-McKay, K. E., Venneri, A., & Ellis, A.W. (2003). *The effect of age of acquisition on word production in Alzheimer's disease.* Paper presented at the Proceedings of the British Psychological Society.

Fox, N. C., & Schott, J. M. (2004). Imaging cerebral atrophy: normal ageing to Alzheimer's disease. *Lancet, 363*(9406), 392-394.

Fox, N. C., Warrington, E. K., Freeborough, P. A., Hartikainen, P., Kennedy, A. M., Stevens, J. M., et al. (1996b). Presymptomatic hippocampal atrophy in Alzheimer's disease: A longitudinal MRI study. *Brain, 119 (Pt 6),* 2001-2007.

Fox, N. C., Warrington, E. K., Stevens, J. M., & Rossor, M. N. (1996a). Atrophy of the hippocampal formation in early familial Alzheimer's disease: A longitudinal MRI study of at-risk members of a family with an amyloid precursor protein 717Val-Gly mutation. *Ann N Y Acad Sci, 777,* 226-232.

Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., et al. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A, 102*(30), 10604-10609.

Frank, R. A., Galasko, D., & Hampel, H. (2003). Biological Markers for therapeutic trials in Alzheimer's disease. Proceeding of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. *Neurobiology of Aging, 24*, 521-536.

Fratiglioni, L., Launer, L. J., Andersen, K., Breteler, M. M., Copeland, J. R., Dartigues, J. F., et al. (2000). Incidence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. *Neurology, 54*(11 Suppl 5), S10-15.

Fratiglioni, L., Viitanen, M., von Strauss, E., Tontodonati, V., Herlitz, A., & Winblad, B. (1997). Very old women at highest risk of dementia and Alzheimer's disease: Incidence data from the Kungsholmen Project, Stockholm. *Neurology, 48*(1), 132-138.

Frisoni, G. B., Testa, C., Zorzan, A., Sabattoli, F., Beltramello, A., Soininen, H., et al. (2002). Detection of grey matter loss in mild Alzheimer's disease with voxel based morphometry. *J Neurol Neurosurg Psychiatry, 73*(6), 657-664.

Fullerton, S. M., Clark, A. G., Weiss, K. M., Nickerson, D. A., Taylor, S. L., Stengard, J. H., et al. (2000). Apolipoprotein E variation at the sequence haplotype level:Implications for the origin and maintenance of a major human polymorphism. *Am J Hum Genet, 67*(4), 881-900.

Funnell, E. (2005). The single case study and Alzheimer-type dementia

In R. G. M. (Eds.) (Ed.), *The Cognitive Neuropsychology of Alzheimer's Disease 2nd edition* (pp. 2-22). Oxford: Oxford University Press.

Furukawa, K., Guo, Q., Schellenberg, G. D., & Mattson, M. P. (1998). Presenilin-1 mutation alters NGF-induced neurite outgrowth, calcium homeostasis, and transcription factor (AP-1) activation in PC12 cells. *J Neurosci Res, 52*(5), 618-624.

Fuso, A., Seminara, L., Cavallaro, R. A., D'Anselmi, F., & Scarpa, S. (2005). S-adenosylmethionine/homocysteine cycle alterations modify DNA methylation status with consequent deregulation of PS1 and BACE and beta-amyloid production. *Mol Cell Neurosci, 28*(1), 195-204.

Ganguli, M., Chandra, V., Kamboh, M. I., Johnston, J. M., Dodge, H. H., Thelma, B. K., et al. (2000). Apolipoprotein E polymorphism and Alzheimer disease: The Indo-US Cross-National Dementia Study. *Arch Neurol, 57*(6), 824-830.

Garrard, P., Maloney, L. M., Hodges, J. R., & Patterson, K. (2005). The effects of very early Alzheimer's disease on the characteristics of writing by a renowned author. *Brain, 128*(Pt 2), 250-260.

Garrard, P., Perry, R., & Hodges, J. R. (1997). Disorders of semantic memory. *J Neurol Neurosurg Psychiatry, 62*(5), 431-435.

Gatz, M., Pedersen, N. L., Berg, S., Johansson, B., Johansson, K., Mortimer, J. A., et al. (1997). Heritability for Alzheimer's disease: The study of dementia in Swedish twins. *J Gerontol A Biol Sci Med Sci, 52*(2), M117-125.

Gearing, M., Mori, H., & Mirra, S. S. (1996). Abeta-peptide length and apolipoprotein E genotype in Alzheimer's disease. *Ann Neurol, 39*(3), 395-399.

Geroldi, C., Pihlajamaki, M., Laakso, M. P., DeCarli, C., Beltramello, A., Bianchetti, A., et al. (1999). APOE-epsilon4 is associated with less frontal and more medial temporal lobe atrophy in AD. *Neurology, 53*(8), 1825-1832.

Geslani, D. M., Tierney, M. C., Herrmann, N., & Szalai, J. P. (2005). Mild cognitive impairment: An operational definition and its conversion rate to Alzheimer's disease. *Dement Geriatr Cogn Disord, 19*(5-6), 383-389.

Giacobini, E. (2000). *Cholinesterases and Cholinesterase Inhibitors.* London, Uk.: Martin Dunitz.

Glenner, G. G. (1989). The pathobiology of Alzheimer's disease. *Annu Rev Med, 40,* 45-51.

Gluckman, P. D., Hanson, M. A., Buklijas, T., Low, F. M., & Beedle, A. S. (2009). Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat Rev Endocrinol, 5*(7), 401-408.

Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., & Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: Identification as the microtubule-associated protein tau. *Proc Natl Acad Sci U S A, 85*(11), 4051-4055.

Goldstein, J. L., & Brown, M. S. (1976). The LDL pathway in human fibroblasts: A receptor-mediated mechanism for the regulation of cholesterol metabolism. *Curr Top Cell Regul, 11*, 147-181.

Gomez-Isla, T., Irizarry, M. C., Mariash, A., Cheung, B., Soto, O., Schrump, S., et al. (2003). Motor dysfunction and gliosis with preserved dopaminergic markers in human alpha-synuclein A30P transgenic mice. *Neurobiol Aging, 24*(2), 245-258.

Gomez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., & Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci, 16*(14), 4491-4500.

Gomez Beldarrain, M., Grafman, J., Pascual-Leone, A., & Garcia-Monco, J. C. (1999). Procedural learning is impaired in patients with prefrontal lesions. *Neurology, 52*(9), 1853-1860.

Gottesman, II, & Gould, T. D. (2003). The endophenotype concept in psychiatry: Etymology and strategic intentions. *Am J Psychiatry, 160*(4), 636-645.

Gourovitch, M. L., Kirkby, B. S., Goldberg, T. E., Weinberger, D. R., Gold, J. M., Esposito, G., et al. (2000). A comparison of rCBF patterns during letter and semantic fluency. *Neuropsychology, 14*(3), 353-360.

Grady, C. L., McIntosh, A. R., Beig, S., Keightley, M. L., Burian, H., & Black, S. E. (2003). Evidence from functional neuroimaging of a compensatory prefrontal network in Alzheimer's disease. *J Neurosci, 23*(3), 986-993.

Green, R. C., Cupples, L. A., Go, R., Benke, K. S., Edeki, T., Griffith, P. A., et al. (2002). Risk of dementia among white and African American relatives of patients with Alzheimer disease. *JAMA, 287*(3), 329-336.

Greenfield, J. P., Leung, L. W., Cai, D., Kaasik, K., Gross, R. S., Rodriguez-Boulan, E., et al. (2002). Estrogen lowers Alzheimer beta-amyloid generation by stimulating trans-Golgi network vesicle biogenesis. *J Biol Chem, 277*(14), 12128-12136.

Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: Evidence from functional MRI. *Proc Natl Acad Sci U S A, 101*(13), 4637-4642.

Grundman, M., Petersen, R. C., Ferris, S. H., Thomas, R. G., Aisen, P. S., Bennett, D. A., et al. (2004). Mild cognitive impairment can be distinguished from

Alzheimer disease and normal aging for clinical trials. *Archives of Neurology, 61*(1), 59-66.

Grundman, M., Petersen, R. C., Ferris, S. H., Thomas, R. G., Aisen, P. S., Bennett, D. A., et al. (2004). Mild cognitive impairment can be distinguished from Alzheimer disease and normal aging for clinical trials. *Arch Neurol, 61*(1), 59-66.

Guo, L., LaDu, M. J., & Van Eldik, L. J. (2004). A dual role for apolipoprotein e in neuroinflammation: Anti- and pro-inflammatory activity. *J Mol Neurosci, 23*(3), 205-212.

Guo, Z., Cupples, L. A., Kurz, A., Auerbach, S. H., Volicer, L., Chui, H., et al. (2000). Head injury and the risk of AD in the MIRAGE study. *Neurology, 54*(6), 1316-1323.

Gurland, B. J., Wilder, D. E., Lantigua, R., Stern, Y., Chen, J., Killeffer, E. H., et al. (1999). Rates of dementia in three ethnoracial groups. *Int J Geriatr Psychiatry, 14*(6), 481-493.

Hariri, A. R., & Weinberger, D. R. (2003). Imaging genomics. *Br Med Bull, 65*, 259-270.

Hakansson, K., Rovio, S., Helkala, E. L., Vilska, A. R., Winblad, B., Soininen, H., et al. (2009). Association between mid-life marital status and cognitive function in later life: Population based cohort study. *BMJ, 339*, b2462.

Hake, S. B., Xiao, A., & Allis, C. D. (2004). Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br J Cancer, 90*(4), 761-769.

Hall, K. S., Gao, S., Unverzagt, F. W., & Hendrie, H. C. (2000). Low education and childhood rural residence: Risk for Alzheimer's disease in African Americans. *Neurology, 54*(1), 95-99.

Hamalainen, A., Grau-Olivares, M., Tervo, S., Niskanen, E., Pennanen, C., Huuskonen, J., et al. (2008). Apolipoprotein E epsilon 4 allele is associated with increased atrophy in progressive mild cognitive impairment: A voxel-based morphometric study. *Neurodegener Dis, 5*(3-4), 186-189.

Hamalainen, A., Tervo, S., Grau-Olivares, M., Niskanen, E., Pennanen, C., Huuskonen, J., et al. (2007a). Voxel-based morphometry to detect brain atrophy in progressive mild cognitive impairment. *Neuroimage, 37*(4), 1122-1131.

Han, S. D., Houston, W. S., Jak, A. J., Eyler, L. T., Nagel, B. J., Fleisher, A. S., et al. (2007). Verbal paired-associate learning by APOE genotype in non-demented older adults: fMRI evidence of a right hemispheric compensatory response. *Neurobiol Aging, 28*(2), 238-247.

Hardy, J. (1997). Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci, 20*(4), 154-159.

Hardy, J. (2009). The amyloid hypothesis for Alzheimer's disease: A critical reappraisal. *J Neurochem, 110*(4), 1129-1134.

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M. L., et al. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet, 41*(10), 1088-1093.

Harris, F. M., Brecht, W. J., Xu, Q., Tesseur, I., Kekonius, L., Wyss-Coray, T., et al. (2003). Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proc Natl Acad Sci U S A, 100*(19), 10966-10971.

Harvan, J. R., & Cotter, V. (2006). An evaluation of dementia screening in the primary care setting. *J Am Acad Nurse Pract, 18*(8), 351-360.

Hashimoto, M., Yasuda, M., Tanimukai, S., Matsui, M., Hirono, N., Kazui, H., et al. (2001). Apolipoprotein E epsilon 4 and the pattern of regional brain atrophy in Alzheimer's disease. *Neurology, 57*(8), 1461-1466.

Hebert, L. E., Beckett, L. A., Scherr, P. A., & Evans, D. A. (2001). Annual incidence of Alzheimer disease in the United States projected to the years 2000 through 2050. *Alzheimer Dis Assoc Disord, 15*(4), 169-173.

Henderson, A. S. H., K. (1992). The epidemiology of dementia and depression in later life. In H. K. Bergener M., S.I. Finkel, & T. Nishimura (Ed.), *Aging and Mental Disorders, International Perspective* (pp. 65-79). New York: Springer Publishing Company.

Henry, J. D., & Crawford, J. R. (2004). A meta-analytic review of verbal fluency performance following focal cortical lesions. *Neuropsychology, 18*(2), 284-295.

Henry, J. D., Crawford, J. R., & Phillips, L. H. (2004). Verbal fluency performance in dementia of the Alzheimer's type: A meta-analysis. *Neuropsychologia, 42*(9), 1212-1222.

Hernandez-Munoz, N., Izura, C., & Ellis, A.W. (2006). Cognitive aspects of lexical availability. *Psychological Bulletin, 133*, 638-650.

Higuchi, M., Arai, H., Nakagawa, T., Higuchi, S., Muramatsu, T., Matsushita, S., et al. (1997). Regional cerebral glucose utilization is modulated by the dosage of apolipoprotein E type 4 allele and alpha1-antichymotrypsin type A allele in Alzheimer's disease. *Neuroreport, 8*(12), 2639-2643.

Hirono, N., Hashimoto, M., Yasuda, M., Kazui, H., & Mori, E. (2003). Accelerated memory decline in Alzheimer's disease with apolipoprotein epsilon4 allele. *J Neuropsychiatry Clin Neurosci, 15*(3), 354-358.

Hirono, N., Mori, E., Yasuda, M., Ishii, K., Ikejiri, Y., Imamura, T., et al. (1998). Lack of association of apolipoprotein E epsilon4 allele dose with cerebral glucose metabolism in Alzheimer disease. *Alzheimer Dis Assoc Disord, 12*(4), 362-367.

Hixson, J. E. (1991). Apolipoprotein E polymorphisms affect atherosclerosis in young males. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Arterioscler Thromb, 11*(5), 1237-1244.

Ho, P. I., Collins, S. C., Dhitavat, S., Ortiz, D., Ashline, D., Rogers, E., et al. (2001). Homocysteine potentiates beta-amyloid neurotoxicity: Role of oxidative stress. *J Neurochem, 78*(2), 249-253.

Hodges, J. R., Erzinclioglu, S., & Patterson, K. (2006). Evolution of cognitive deficits and conversion to dementia in patients with mild cognitive impairment: A very-long-term follow-up study. *Dement Geriatr Cogn Disord, 21*(5-6), 380-391.

Hodges, J. R., & Patterson, K. (1995). Is semantic memory consistently impaired early in the course of Alzheimer's disease? Neuroanatomical and diagnostic implications. *Neuropsychologia, 33*(4), 441-459.

Hodges, J. R., Patterson, K., Graham, N., & Dawson, K. (1996). Naming and knowing in dementia of Alzheimer's type. *Brain Lang, 54*(2), 302-325.

Hodges, J. R., Salmon, D. P., & Butters, N. (1991). The nature of the naming deficit in Alzheimer's and Huntington's disease. *Brain, 114 (Pt 4)*, 1547-1558.

Hodges, J. R., Salmon, D. P., & Butters, N. (1992). Semantic memory impairment in Alzheimer's disease: Failure of access or degraded knowledge? *Neuropsychologia, 30*(4), 301-314.

Hodgson, C., & Ellis, A. W. (1998). Last in, first to go: age of acquisition and naming in the elderly. *Brain Lang, 64*(1), 146-163.

Holmes, S. J., Fitch, F. J., & Ellis, A. W. (2006). Age of acquisition affects object recognition and naming in patients with Alzheimer's disease. *J Clin Exp Neuropsychol, 28*(6), 1010-1022.

Holmes, S. J., Jane Fitch, F., & Ellis, A. W. (2006). Age of acquisition affects object recognition and naming in patients with Alzheimer's disease. *J Clin Exp Neuropsychol, 28*(6), 1010-1022.

Holtzman, D. M., Bales, K. R., Tenkova, T., Fagan, A. M., Parsadanian, M., Sartorius, L. J., et al. (2000a). Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A, 97*(6), 2892-2897.

Holtzman, D. M., Fagan, A. M., Mackey, B., Tenkova, T., Sartorius, L., Paul, S. M., et al. (2000b). Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. *Ann Neurol, 47*(6), 739-747.

Holzer, M., Holzapfel, H. P., Zedlick, D., Bruckner, M. K., & Arendt, T. (1994). Abnormally phosphorylated tau protein in Alzheimer's disease: Heterogeneity of individual regional distribution and relationship to clinical severity. *Neuroscience, 63*(2), 499-516.

Honea, R. A., Swerdlow, R. H., Vidoni, E. D., Goodwin, J., & Burns, J. M. (2010). Reduced gray matter volume in normal adults with a maternal family history of Alzheimer disease. *Neurology, 74*(2), 113-120.

Horsburgh, K., Cole, G. M., Yang, F., Savage, M. J., Greenberg, B. D., Gentleman, S. M., et al. (2000). Beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury. *Neuropathol Appl Neurobiol, 26*(2), 124-132.

Howard, D., & Patterson, K. (1992). *Pyramids and Palm Trees A Test of Semantc Access From Pictures and words*: Thames Valley Publishing Company, Bury.

Hsieh, J., & Gage, F. H. (2005). Chromatin remodeling in neural development and plasticity. *Curr Opin Cell Biol, 17*(6), 664-671.

Hsiung, G. Y., Sadovnick, A. D., & Feldman, H. (2004). Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: Data from the Canadian Study of Health and Aging. *CMAJ, 171*(8), 863-867.

Hua, X., Leow, A. D., Parikshak, N., Lee, S., Chiang, M. C., Toga, A. W., et al. (2008a). Tensor-based morphometry as a neuroimaging biomarker for Alzheimer's disease: An MRI study of 676 AD, MCI, and normal subjects. *Neuroimage, 43*(3), 458-469.

Huang, H. C., & Jiang, Z. F. (2009). Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: Relationship and links in Alzheimer's disease. *J Alzheimers Dis, 16*(1), 15-27.

Hubacek, J. A., Pitha, J., Skodova, Z., Adamkova, V., Lanska, V., & Poledne, R. (2001). A possible role of apolipoprotein E polymorphism in predisposition to higher education. *Neuropsychobiology, 43*(3), 200-203.

Huff, F. J., Corkin, S., & Growdon, J. H. (1986). Semantic impairment and anomia in Alzheimer's disease. *Brain Lang, 28*(2), 235-249.

Hughes, C. P., Berg, L., Danziger, W. L., Coben, L. A., & Martin, R. L. (1982). A new clinical scale for the staging of dementia. *Br J Psychiatry, 140*, 566-572.

The Human Gene Mutation Database. (2008). Retrieved 15-01-2010: http://www.hgmd.org

Huriletemuer, Wang, B., Wang, J., Wang, G., Zhang, C., Zhao, S., et al. (2010). APOE epsilon4 is a high-risk factor for Alzheimer's disease in the Mongolian population. *J Neurol Sci, 288*(1-2), 167-169.

Innerarity, T. L., & Mahley, R. W. (1978). Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoproteins. *Biochemistry, 17*(8), 1440-1447.

Innerarity, T. L., Pitas, R. E., & Mahley, R. W. (1979). Binding of arginine-rich (E) apoprotein after recombination with phospholipid vesicles to the low density lipoprotein receptors of fibroblasts. *J Biol Chem, 254*(10), 4186-4190.

Itzhaki, R. F., Lin, W. R., Shang, D., Wilcock, G. K., Faragher, B., & Jamieson, G. A. (1997). Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *Lancet, 349*(9047), 241-244.

Jack, C. R., Jr., Bernstein, M. A., Fox, N. C., Thompson, P., Alexander, G., Harvey, D., et al. (2008). The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. *J Magn Reson Imaging, 27*(4), 685-691.

Jack, C. R., Jr., Petersen, R. C., Xu, Y. C., O'Brien, P. C., Smith, G. E., Ivnik, R. J., et al. (1999). Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology, 52*(7), 1397-1403.

Jack, C. R., Jr., Petersen, R. C., Xu, Y. C., O'Brien, P. C., Waring, S. C., Tangalos, E. G., et al. (1998). Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. *Ann Neurol, 43*(3), 303-310.

Jack, C. R., Jr., Petersen, R. C., Xu, Y. C., O'Brien, P. C., Waring, S. C., Tangalos, E. G., et al. (1998b). Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. *Ann Neurol, 43*(3), 303-310.

Jack, J., & Myette, G. (1997). The genes raw and ribbon are required for proper shape of tubular epithelial tissues in Drosophila. *Genetics, 147*(1), 243-253.

Jacobs, D. M., Sano, M., Dooneief, G., Marder, K., Bell, K. L., & Stern, Y. (1995a). Neuropsychological detection and characterization of preclinical Alzheimer's disease. *Neurology, 45*(5), 957-962.

Jellinger, K. A., Paulus, W., Wrocklage, C., & Litvan, I. (2001). Effects of closed traumatic brain injury and genetic factors on the development of Alzheimer's disease. *Eur J Neurol, 8*(6), 707-710.

Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C., et al. (2004). Increased hippocampal neurogenesis in Alzheimer's disease. *Proc Natl Acad Sci U S A, 101*(1), 343-347.

Johnson, J. K., Vogt, B. A., Kim, R., Cotman, C. W., & Head, E. (2004). Isolated executive impairment and associated frontal neuropathology. *Dement Geriatr Cogn Disord, 17*(4), 360-367.

Johnston, R. A., & Barry, C. (2006). Age of acquisition and lexical processing. *Visual Cognition, 13*, 789-845.

Johnstone, B., Hogg, J. R., Schopp, L. H., Kapila, C., & Edwards, S. (2002). Neuropsychological deficit profiles in senile dementia of the Alzheimer's type. *Arch Clin Neuropsychol, 17*(3), 273-281.

Jones, S., Laukka, E. J., & Backman, L. (2006). Differential verbal fluency deficits in the preclinical stages of Alzheimer's disease and vascular dementia. *Cortex, 42*(3), 347-355.

Jost, J. P., & Jost, Y. C. (1995). Mechanism of active DNA demethylation during embryonic development and cellular differentiation in vertebrates. *Gene, 157*(1-2), 265-266.

Joubert, S., Felician, O., Barbeau, E. J., Didic, M., Poncet, M., & Ceccaldi, M. (2008). Patterns of semantic memory impairment in mild cognitive impairment. *Behavioural Neurology, 19*(1-2), 35-40.

Juottonen, K., Laakso, M. P., Insausti, R., Lehtovirta, M., Pitkanen, A., Partanen, K., et al. (1998). Volumes of the entorhinal and perirhinal cortices in Alzheimer's disease. *Neurobiol Aging, 19*(1), 15-22.

Kalaria, R. N., Maestre, G. E., Arizaga, R., Friedland, R. P., Galasko, D., Hall, K., et al. (2008). Alzheimer's disease and vascular dementia in developing countries: Prevalence, management, and risk factors. *Lancet Neurology, 7*(9), 812-826.

Karas, G., Sluimer, J., Goekoop, R., van der Flier, W., Rombouts, S. A., Vrenken, H., et al. (2008). Amnestic mild cognitive impairment: Structural MR imaging findings predictive of conversion to Alzheimer disease. *AJNR Am J Neuroradiol, 29*(5), 944-949.

Karas, G. B., Scheltens, P., Rombouts, S. A., Visser, P. J., van Schijndel, R. A., Fox, N. C., et al. (2004). Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. *Neuroimage, 23*(2), 708-716.

Karlinsky, H., Vaula, G., Haines, J. L., Ridgley, J., Bergeron, C., Mortilla, M., et al. (1992). Molecular and Prospective Phenotypic Characterization of a Pedigree with Familial Alzheimers-Disease and a Missense Mutation in Codon 717 of the Beta-Amyloid Precursor Protein Gene. *Neurology, 42*(8), 1445-1453.

Karrasch, M., Sinerva, E., Gronholm, P., Rinne, J., & Laine, M. (2005). CERAD test performances in amnestic mild cognitive impairment and Alzheimer's disease. *Acta Neurologica Scandinavica, 111*(3), 172-179.

Katsuya, T., Baba, S., Ishikawa, K., Mannami, T., Fu, Y., Inamoto, N., et al. (2002). Epsilon 4 allele of apolipoprotein E gene associates with lower blood pressure in young Japanese subjects: The Suita Study. *J Hypertens, 20*(10), 2017-2021.

Katzman, R. (1993). Education and the Prevalence of Dementia and Alzheimers-Disease. *Neurology, 43*(1), 13-20.

Kaufman, Y., Heled, M., Perk, J., Razin, A., & Shemer, R. (2009). Protein-binding elements establish in the oocyte the primary imprint of the Prader-Willi/Angelman syndromes domain. *Proceedings of the National Academy of Sciences of the United States of America, 106*(25), 10242-10247.

Kaufman, A. S., McLean, J. E., & Reynolds, C. R. (1988). Sex, race, residence, region, and education differences on the 11 WAIS-R subtests. *J Clin Psychol, 44*(2), 231-248

Khachaturian, A. S., Corcoran, C. D., Mayer, L. S., Zandi, P. P., & Breitner, J. C. (2004). Apolipoprotein E epsilon4 count affects age at onset of Alzheimer disease, but not lifetime susceptibility: The Cache County Study. *Arch Gen Psychiatry, 61*(5), 518-524.

Kim, J., Basak, J. M., & Holtzman, D. M. (2009). The role of apolipoprotein E in Alzheimer's disease. *Neuron, 63*(3), 287-303.

Kitamura, H. W., Hamanaka, H., Watanabe, M., Wada, K., Yamazaki, C., Fujita, S. C., et al. (2004). Age-dependent enhancement of hippocampal long-term potentiation in knock-in mice expressing human apolipoprotein E4 instead of mouse apolipoprotein E. *Neurosci Lett, 369*(3), 173-178.

Kivipelto, M., Helkala, E. L., Hanninen, T., Laakso, M. P., Hallikainen, M., Alhainen, K., et al. (2001). Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. *Neurology, 56*(12), 1683-1689.

Knopman, D. S., DeKosky, S. T., Cummings, J. L., Chui, H., Corey-Bloom, J., Relkin, N., et al. (2001). Practice parameter: Diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology, 56*(9), 1143-1153.

Kremin, H., Perrier, D., De Wilde, M., Dordain, M., Le Bayon, A., Gatignol, P., et al. (2001). Factors predicting success in picture naming in Alzheimer's disease and primary progressive aphasia. *Brain Cogn, 46*(1-2), 180-183.

Kukull, W. A., Higdon, R., Bowen, J. D., McCormick, W. C., Teri, L., Schellenberg, G. D., et al. (2002). Dementia and Alzheimer disease incidence: A prospective cohort study. *Arch Neurol, 59*(11), 1737-1746.

Kuwabara, T., Hsieh, J., Nakashima, K., Taira, K., & Gage, F. H. (2004). A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell, 116*(6), 779-793.

Lahiri, D. K., Farlow, M. R., Sambamurti, K., Greig, N. H., Giacobini, E., & Schneider, L. S. (2003). A critical analysis of new molecular targets and strategies for drug developments in Alzheimer's disease. *Curr Drug Targets, 4*(2), 97-112.

Lahiri, D. K., Maloney, B., & Zawia, N. H. (2009). The LEARn model: An epigenetic explanation for idiopathic neurobiological diseases. *Mol Psychiatry, 14*(11), 992-1003.

Lambert, J. C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., et al. (2009). Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet, 41*(10), 1094-1099.

Lambon Ralph, M. A., Patterson, K., & Hodges, J. R. (1997). The relationship between naming and semantic knowledge for different categories in dementia of Alzheimer's type. *Neuropsychologia, 35*(9), 1251-1260.

Lanahan, A., Lyford, G., Stevenson, G. S., Worley, P. F., & Barnes, C. A. (1997). Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. *J Neurosci, 17*(8), 2876-2885.

Lange, K. L., Bondi, M. W., Salmon, D. P., Galasko, D., Delis, D. C., Thomas, R. G., et al. (2002). Decline in verbal memory during preclinical Alzheimer's disease: Examination of the effect of APOE genotype. *J Int Neuropsychol Soc, 8*(7), 943-955.

Lanz, T. A., Carter, D. B., & Merchant, K. M. (2003). Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. *Neurobiol Dis, 13*(3), 246-253.

Larochelle, S., Richard, S., & Soulieres, I. (2000). What some effects might not be: The time to verify membership in "well-defined" categories. *Quarterly Journal of Experimental Psychology Section a-Human Experimental Psychology, 53*(4), 929-961.

Launer, L. J., & Brock, D. B. (2004). Population-based studies of AD: Message and methods: An epidemiologic view. *Stat Med, 23*(2), 191-197.

Lehmann, D. J., Refsum, H., Nurk, E., Warden, D. R., Tell, G. S., Vollset, S. E., et al. (2006). Apolipoprotein E epsilon4 and impaired episodic memory in community-dwelling elderly people: A marked sex difference. The Hordaland Health Study. *J Neurol Neurosurg Psychiatry, 77*(8), 902-908.

Lehtovirta, M., Laakso, M. P., Frisoni, G. B., & Soininen, H. (2000). How does the apolipoprotein E genotype modulate the brain in aging and in Alzheimer's disease? A review of neuroimaging studies. *Neurobiol Aging*, *21*(2), 293-300.

Lehtovirta, M., Laakso, M. P., Soininen, H., Helisalmi, S., Mannermaa, A., Helkala, E. L., et al. (1995). Volumes of hippocampus, amygdala and frontal lobe in Alzheimer patients with different apolipoprotein E genotypes. *Neuroscience, 67*(1), 65-72.

Lehtovirta, M., Soininen, H., Laakso, M. P., Partanen, K., Helisalmi, S., Mannermaa, A., et al. (1996). SPECT and MRI analysis in Alzheimer's disease: Relation to apolipoprotein E epsilon 4 allele. *J Neurol Neurosurg Psychiatry, 60*(6), 644-649.

Lemaitre, H., Crivello, F., Dufouil, C., Grassiot, B., Tzourio, C., Alperovitch, A., et al. (2005). No epsilon4 gene dose effect on hippocampal atrophy in a large MRI database of healthy elderly subjects. *Neuroimage*, *24*(4), 1205-1213.

Lessov-Schlaggar, C. N., Swan, G. E., Reed, T., Wolf, P. A., & Carmelli, D. (2007). Longitudinal genetic analysis of executive function in elderly men. *Neurobiol Aging*, *28*(11), 1759-1768.

Levi, O., & Michaelson, D. M. (2007). Environmental enrichment stimulates neurogenesis in apolipoprotein E3 and neuronal apoptosis in apolipoprotein E4 transgenic mice. *J Neurochem, 100*(1), 202-210.

Lezak, M. D. H., D.B., Loring, D.W., Hannay, H.J. and Fischer, J.S., (2004). *Neuropsychological assessment (4th ed.)*. New York.

Li, H., Wetten, S., Li, L., St Jean, P. L., Upmanyu, R., Surh, L., et al. (2008). Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. *Arch Neurol, 65*(1), 45-53.

Lind, J., Larsson, A., Persson, J., Ingvar, M., Nilsson, L. G., Backman, L., et al. (2006). Reduced hippocampal volume in non-demented carriers of the apolipoprotein E epsilon4: Relation to chronological age and recognition memory. *Neurosci Lett, 396*(1), 23-27.

Lindsay, J., Laurin, D., Verreault, R., Hebert, R., Helliwell, B., Hill, G. B., et al. (2002). Risk factors for Alzheimer's disease: A prospective analysis from the Canadian Study of Health and Aging. *Am J Epidemiol, 156*(5), 445-453.

Liu, E. T. (2008). Functional genomics of cancer. *Curr Opin Genet Dev, 18*(3), 251-256.

Liu, H. C., Hong, C. J., Wang, S. J., Fuh, J. L., Wang, P. N., Shyu, H. Y., et al. (1999). ApoE genotype in relation to AD and cholesterol: A study of 2,326 Chinese adults. *Neurology, 53*(5), 962-966.

Liu, L., Wylie, R. C., Andrews, L. G., & Tollefsbol, T. O. (2003). Aging, cancer and nutrition: The DNA methylation connection. *Mech Ageing Dev, 124*(10-12), 989-998.

Luchsinger, J. A., & Gustafson, D. R. (2009). Adiposity and Alzheimer's disease. *Curr Opin Clin Nutr Metab Care, 12*(1), 15-21.

Lusis, A. J., Heinzmann, C., Sparkes, R. S., Scott, J., Knott, T. J., Geller, R., et al. (1986). Regional mapping of human chromosome 19: Organization of genes for plasma lipid transport (APOC1, -C2, and -E and LDLR) and the genes C3, PEPD, and GPI. *Proc Natl Acad Sci U S A, 83*(11), 3929-3933.

Maeda, T., Guan, J. Z., Oyama, J., Higuchi, Y., & Makino, N. (2009). Aging-associated alteration of subtelomeric methylation in Parkinson's disease. *J Gerontol A Biol Sci Med Sci, 64*(9), 949-955.

Mahley, R. W. (1983). Apolipoprotein E and cholesterol metabolism. *Klin Wochenschr, 61*(5), 225-232.

Mahley, R. W. (1988). Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. *Science, 240*(4852), 622-630.

Mahley, R. W., & Huang, Y. (2009b). Alzheimer disease: Multiple causes, multiple effects of apolipoprotein E4, and multiple therapeutic approaches. *Ann Neurol, 65*(6), 623-625.

Mahley, R. W., Huang, Y., & Rall, S. C., Jr. (1999). Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia).Questions, quandaries, and paradoxes. *J Lipid Res, 40*(11), 1933-1949.

Mahley, R. W., & Rall, S. C., Jr. (2000). Apolipoprotein E: Far more than a lipid transport protein. *Annu Rev Genomics Hum Genet, 1*, 507-537.

Mahley, R. W., Weisgraber, K. H., & Huang, Y. (2006). Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A, 103*(15), 5644-5651.

Mahley, R. W., Weisgraber, K. H., & Huang, Y. (2009a). Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res, 50* S183-188.

Manipalviratn, S., DeCherney, A., & Segars, J. (2009). Imprinting disorders and assisted reproductive technology. *Fertility and Sterility, 91*(2), 305-315.

Mann, D. M., Lincoln, J., Yates, P. O., Stamp, J. E., & Toper, S. (1980). Changes in the monoamine containing neurones of the human CNS in senile dementia. *Br J Psychiatry, 136*, 533-541.

Manns, J. R., & Squire, L. R. (2001). Perceptual learning, awareness, and the hippocampus. *Hippocampus, 11*(6), 776-782.

Marczinski, C. A., & Kertesz, A. (2006). Category and letter fluency in semantic dementia, primary progressive aphasia, and Alzheimer's disease. *Brain Lang, 97*(3), 258-265.

Marques, J. F., Fonseca, F. L., Morais, A. S., & Pinto, I. A. (2007). Estimated age of acquisition norms for 834 Portuguese nouns and their relation with other psycholinguistic variables. *Behav Res Methods, 39*(3), 439-444.

Marquis, S., Moore, M. M., Howieson, D. B., Sexton, G., Payami, H., Kaye, J. A., et al. (2002). Independent predictors of cognitive decline in healthy elderly persons. *Arch Neurol, 59*(4), 601-606.

Martins, C. A., Oulhaj, A., de Jager, C. A., & Williams, J. H. (2005). APOE alleles predict the rate of cognitive decline in Alzheimer disease: A nonlinear model. *Neurology, 65*(12), 1888-1893.

Mastroeni, D., Grover, A., Delvaux, E., Whiteside, C., Coleman, P. D., & Rogers, J. (2008). Epigenetic changes in Alzheimer's disease: Decrements in DNA methylation. *Neurobiol Aging*.

Mastroeni, D., McKee, A., Grover, A., Rogers, J., & Coleman, P. D. (2009). Epigenetic differences in cortical neurons from a pair of monozygotic twins discordant for Alzheimer's disease. *PLoS One, 4*(8), e6617.

Masur, D. M., Sliwinski, M., Lipton, R. B., Blau, A. D., & Crystal, H. A. (1994). Neuropsychological prediction of dementia and the absence of dementia in healthy elderly persons. *Neurology, 44*(8), 1427-1432

Mauch, D. H., Nagler, K., Schumacher, S., Goritz, C., Muller, E. C., Otto, A., et al. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. *Science, 294*(5545), 1354-1357.

Mayeux, R. (2003a). Epidemiology of neurodegeneration. *Annu Rev Neurosci, 26*, 81-104.

Mayeux, R., Lee, J. H., Romas, S. N., Mayo, D., Santana, V., Williamson, J., et al. (2002). Chromosome-12 mapping of late-onset Alzheimer disease among Caribbean Hispanics. *Am J Hum Genet, 70*(1), 237-243.

Mayeux, R., & Small, S. A. (2000). Finding the beginning or predicting the future? *Arch Neurol, 57*(6), 783-784.

Mayeux, R., Small, S. A., Tang, M., Tycko, B., & Stern, Y. (2001). Memory performance in healthy elderly without Alzheimer's disease: Effects of time and apolipoprotein-E. *Neurobiol Aging, 22*(4), 683-689.

Mayeux, R., Stern, Y., Ottman, R., Tatemichi, T. K., Tang, M. X., Maestre, G., et al. (1993). The Apolipoprotein Epsilon-4 Allele in Patients with Alzheimers-Disease. *Annals of Neurology, 34*(5), 752-754.

McKeith, I. G., Galasko, D., Kosaka, K., Perry, E. K., Dickson, D. W., Hansen, L. A., et al. (1996). Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): Report of the consortium on DLB international workshop. *Neurology, 47*(5), 1113-1124.

Mckhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical Diagnosis of Alzheimers Disease Report of the Nincds Adrda

Work Group under the Auspices of Department-of-Health-and-Human-Services Task Force on Alzheimers Disease. *Neurology, 34*(7), 939-944.

McNamara, M. J., Gomez-Isla, T., & Hyman, B. T. (1998). Apolipoprotein E genotype and deposits of Abeta40 and Abeta42 in Alzheimer disease. *Arch Neurol, 55*(7), 1001-1004.

Menenti, L., & Burani, C. (2007). What causes the effect of age of acquisition in lexical processing? *Q J Exp Psychol (Colchester), 60*(5), 652-660.

Merchant, C., Tang, M. X., Albert, S., Manly, J., Stern, Y., & Mayeux, R. (1999). The influence of smoking on the risk of Alzheimer's disease. *Neurology, 52*(7), 1408-1412.

Mesa MD., F.-N. L., Cacabelos R. (1999). Association of genetic risk factors in Alzheimer's disease. *Annual of General Psychiatry, 7*, 35-45.

Mesulam, M. M. (2000). A plasticity-based theory of the pathogenesis of Alzheimer's disease. *Ann N Y Acad Sci, 924*, 42-52.

Meyer, M. R., Tschanz, J. T., Norton, M. C., Welsh-Bohmer, K. A., Steffens, D. C., Wyse, B. W., et al. (1998). APOE genotype predicts when--not whether--one is predisposed to develop Alzheimer disease. *Nat Genet, 19*(4), 321-322.

Miech, R. A., Breitner, J. C., Zandi, P. P., Khachaturian, A. S., Anthony, J. C., & Mayer, L. (2002). Incidence of AD may decline in the early 90s for men, later for women: The Cache County study. *Neurology, 58*(2), 209-218.

Millard, P. H. (1981). Last scene of all. *Br Med J (Clin Res Ed), 283*(6306), 1559-1560.

Miller, K. J., Rogers, S. A., Siddarth, P., & Small, G. W. (2005). Object naming and semantic fluency among individuals with genetic risk for Alzheimer's disease. *Int J Geriatr Psychiatry, 20*(2), 128-136.

Minoshima, S., Frey, K. A., Koeppe, R. A., Foster, N. L., & Kuhl, D. E. (1995). A diagnostic approach in Alzheimer's disease using three-dimensional stereotactic surface projections of fluorine-18-FDG PET. *J Nucl Med, 36*(7), 1238-1248.

Minoshima, S., Giordani, B., Berent, S., Frey, K. A., Foster, N. L., & Kuhl, D. E. (1997). Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann Neurol, 42*(1), 85-94.

Misra, C., Fan, Y., & Davatzikos, C. (2009). Baseline and longitudinal patterns of brain atrophy in MCI patients, and their use in prediction of short-term conversion to AD: Results from ADNI. *Neuroimage, 44*(4), 1415-1422.

Moceri, V. M., Kukull, W. A., Emanuel, I., van Belle, G., & Larson, E. B. (2000). Early-life risk factors and the development of Alzheimer's disease. *Neurology, 54*(2), 415-420.

Modrego, P. J. (2009). Depression in the prodromal phase of Alzheimer disease and the reverse causal hypothesis. *Arch Gen Psychiatry, 66*(1), 107.

Mondadori, C. R., de Quervain, D. J., Buchmann, A., Mustovic, H., Wollmer, M. A., Schmidt, C. F., et al. (2007). Better memory and neural efficiency in young apolipoprotein E epsilon4 carriers. *Cereb Cortex, 17*(8), 1934-1947.

Monsch, A. U., Bondi, M. W., Butters, N., Salmon, D. P., Katzman, R., & Thal, L. J. (1992). Comparisons of verbal fluency tasks in the detection of dementia of the Alzheimer type. *Arch Neurol, 49*(12), 1253-1258.

Morgan, A. R., Turic, D., Jehu, L., Hamilton, G., Hollingworth, P., Moskvina, V., et al. (2007). Association studies of 23 positional/functional candidate genes on chromosome 10 in late-onset Alzheimer's disease. *Am J Med Genet B Neuropsychiatr Genet, 144B*(6), 762-770.

Mori, H. (2000). Untangling Alzheimer's disease from fibrous lesions of neurofibrillary tangles and senile plaques. *Neuropathology, 20 Suppl*, S55-60.

Morishima-Kawashima, M., Oshima, N., Ogata, H., Yamaguchi, H., Yoshimura, M., Sugihara, S., et al. (2000). Effect of apolipoprotein E allele epsilon4 on the initial phase of amyloid beta-protein accumulation in the human brain. *Am J Pathol, 157*(6), 2093-2099.

Morra, J. H., Tu, Z. W., Apostolova, L. G., Green, A. E., Avedissian, C., Madsen, S. K., et al. (2009). Automated 3D Mapping of Hippocampal Atrophy and Its Clinical Correlates in 400 Subjects with Alzheimer's Disease, Mild Cognitive Impairment, and Elderly Controls. *Human Brain Mapping, 30*(9), 2766-2788.

Morrison, C. M., Chappell, T. D., & Ellis, A. W. (1997). Age of acquisition norms for a large set of object names and their relation to adult estimates and other variables. *Quarterly Journal of Experimental Psychology 50A*, 528-559.

Mosconi, L., Nacmias, B., Sorbi, S., De Cristofaro, M. T., Fayazz, M., Tedde, A., et al. (2004). Brain metabolic decreases related to the dose of the ApoE e4 allele in Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 75*(3), 370-376.

Mueller, S. G., Schuff, N., Raptentsetsang, S., Elman, J., & Weiner, M. W. (2008). Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage, 42*(1), 42-48.

Mukaetova-Ladinska, E. B., Harrington, C. R., Roth, M., & Wischik, C. M. (1997). Presence of the apolipoprotein E type epsilon 4 allele is not associated with neurofibrillary pathology or biochemical changes to tau protein. *Dement Geriatr Cogn Disord, 8*(5), 288-295.

Mullan, M., & Crawford, F. (1993). Genetic and molecular advances in Alzheimer's disease. *Trends Neurosci, 16*(10), 398-403.

Mungas, D., Jagust, W. J., Reed, B. R., Kramer, J. H., Weiner, M. W., Schuff, N., et al. (2001). MRI predictors of cognition in subcortical ischemic vascular disease and Alzheimer's disease. *Neurology, 57*(12), 2229-2235.

Murphy, G. M., Jr., Taylor, J., Kraemer, H. C., Yesavage, J., & Tinklenberg, J. R. (1997). No association between apolipoprotein E epsilon 4 allele and rate of decline in Alzheimer's disease. *Am J Psychiatry, 154*(5), 603-608.

Murphy, K. J., Rich, J. B., & Troyer, A. K. (2006). Verbal fluency patterns in amnestic mild cognitive impairment are characteristic of Alzheimer's type dementia. *J Int Neuropsychol Soc, 12*(4), 570-574.

Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E., & Ikeda, K. (1991). Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Research, 541*(1), 163-166.

Nathan, B. P., Jiang, Y., Wong, G. K., Shen, F., Brewer, G. J., & Struble, R. G. (2002). Apolipoprotein E4 inhibits, and apolipoprotein E3 promotes neurite outgrowth in cultured adult mouse cortical neurons through the low-density lipoprotein receptor-related protein. *Brain Research, 928*(1-2), 96-105.

Negash, S., Petersen, L. E., Geda, Y. E., Knopman, D. S., Boeve, B. F., Smith, G. E., et al. (2007). Effects of ApoE genotype and mild cognitive impairment on implicit learning. *Neurobiol Aging, 28*(6), 885-893.

Nestor, P. J., Fryer, T. D., Smielewski, P., & Hodges, J. R. (2003). Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. *Annals of Neurology, 54*(3), 343-351.

Neumeister, P., Albanese, C., Balent, B., Greally, J., & Pestell, R. G. (2002). Senescence and epigenetic dysregulation in cancer. *Int J Biochem Cell Biol, 34*(11), 1475-1490.

Newman, S. K., Warrington, E. K., Kennedy, A. M., & Rossor, M. N. (1994). The Earliest Cognitive Change in a Person with Familial Alzheimers-Disease - Presymptomatic Neuropsychological Features in a Pedigree with Familial Alzheimers Disease Confirmed at Necropsy. *Journal of Neurology Neurosurgery and Psychiatry, 57*(8), 967-972.

Nilsson, L. G., Adolfsson, R., Backman, L., Cruts, M., Nyberg, L., Small, B. J., et al. (2006). The influence of APOE status on episodic and semantic memory: Data from a population-based study. *Neuropsychology, 20*(6), 645-657.

Nilsson, L. N. G., Arendash, G. W., Leighty, R. E., Costa, D. A., Low, M. A., Garcia, M. F., et al. (2004). Cognitive impairment in PDAPP mice depends on ApoE and ACT-catalyzed amyloid formation. *Neurobiology of Aging, 25*(9), 1153-1167.

Nordberg, A., Hellstrom-Lindahl, E., Lee, M., Johnson, M., Mousavi, M., Hall, R., et al. (2002). Chronic nicotine treatment reduces beta-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw). *J Neurochem, 81*(3), 655-658.

Ogunniyi, A., Baiyewu, O., Gureje, O., Hall, K. S., Unverzagt, F., Siu, S. H., et al. (2000). Epidemiology of dementia in Nigeria: Results from the Indianapolis-Ibadan study. *Eur J Neurol, 7*(5), 485-490.

Ohm, T. G., Muller, H., Braak, H., & Bohl, J. (1995). Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. *Neuroscience, 64*(1), 209-217.

Ohm, T. G., Scharnagl, H., Marz, W., & Bohl, J. (1999). Apolipoprotein E isoforms and the development of low and high Braak stages of Alzheimer's disease-related lesions. *Acta Neuropathol, 98*(3), 273-280.

Olaisen, B., Teisberg, P., & Gedde-Dahl, T., Jr. (1982). The locus for apolipoprotein E (apoE) is linked to the complement component C3 (C3) locus on chromosome 19 in man. *Hum Genet, 62*(3), 233-236.

Oldfield, R. C. (1966). Things, words and the brain. *Quarterly Journal of Experimental Psychology, 18*(4), 340-353.

Orgogozo, J. M., Dartigues, J. F., Lafont, S., Letenneur, L., Commenges, D., Salamon, R., et al. (1997). Wine consumption and dementia in the elderly: A prospective community study in the Bordeaux area. *Rev Neurol (Paris), 153*(3), 185-192.

Oria, R. B., Patrick, P. D., Zhang, H., Lorntz, B., de Castro Costa, C. M., Brito, G. A., et al. (2005). APOE4 protects the cognitive development in children with heavy diarrhea burdens in Northeast Brazil. *Pediatr Res, 57*(2), 310-316.

Ostberg, P., Fernaeus, S. E., Hellstrom, K., Bogdanovic, N., & Wahlund, L. O. (2005). Impaired verb fluency: A sign of mild cognitive impairment. *Brain Lang, 95*(2), 273-279.

Ott, A., Slooter, A. J., Hofman, A., van Harskamp, F., Witteman, J. C., Van Broeckhoven, C., et al. (1998). Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: The Rotterdam Study. *Lancet, 351*(9119), 1840-1843.

Paulesu, E., Goldacre, B., Scifo, P., Cappa, S. F., Gilardi, M. C., Castiglioni, I., et al. (1997). Functional heterogeneity of left inferior frontal cortex as revealed by fMRI. *Neuroreport, 8*(8), 2011-2017.

Payami, H., Zareparsi, S., Montee, K. R., Sexton, G. J., Kaye, J. A., Bird, T. D., et al. (1996). Gender difference in apolipoprotein E-associated risk for familial Alzheimer disease: A possible clue to the higher incidence of Alzheimer disease in women. *Am J Hum Genet, 58*(4), 803-811.

Pendleton, N., Payton, A., van den Boogerd, E. H., Holland, F., Diggle, P., Rabbitt, P. M., et al. (2002). Apolipoprotein E genotype does not predict decline in intelligence in healthy older adults. *Neurosci Lett, 324*(1), 74-76.

Pennanen, C., Testa, C., Boccardi, M., Laakso, M. P., Hallikainen, M., Helkala, E. L., et al. (2006). The effect of apolipoprotein polymorphism on brain in mild cognitive impairment: A voxel-based morphometric study. *Dementia and Geriatric Cognitive Disorders, 22*(1), 60-66.

Pennanen, C., Testa, C., Laakso, M. P., Hallikainen, M., Helkala, E. L., Hanninen, T., et al. (2005). A voxel based morphometry study on mild cognitive impairment. *J Neurol Neurosurg Psychiatry, 76*(1), 11-14.

Pericak-Vance, M. A., Grubber, J., Bailey, L. R., Hedges, D., West, S., Kemmerer, B., et al. (2000). Genomic screen of 739 sibpairs with late onset Alzheimer diease. *American Journal of Human Genetics, 67*(4), 48-48.

Permanne, B., Perez, C., Soto, C., Frangione, B., & Wisniewski, T. (1997). Detection of apolipoprotein E/dimeric soluble amyloid beta complexes in Alzheimer's disease brain supernatants. *Biochem Biophys Res Commun, 240*(3), 715-720.

Perry, R. J., & Hodges, J. R. (2000). Fate of patients with questionable (very mild) Alzheimer's disease: Longitudinal profiles of individual subjects' decline. *Dementia and Geriatric Cognitive Disorders, 11*(6), 342-349.

Pestell, S., Shanks, M. F., Warrington, J., & Venneri, A. (2000). Quality of spelling breakdown in Alzheimer's disease is independent of disease progression. *J Clin Exp Neuropsychol, 22*(5), 599-612.

Petersen, R. C., Smith, G. E., Ivnik, R. J., Tangalos, E. G., Schaid, D. J., Thibodeau, S. N., et al. (1995). Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. *JAMA, 273*(16), 1274-1278.

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. *Arch Neurol, 56*(3), 303-308.

Petersen, R. C., Stevens, J. C., Ganguli, M., Tangalos, E. G., Cummings, J. L., & DeKosky, S. T. (2001). Practice parameter: early detection of dementia: Mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology, 56*(9), 1133-1142.

Petrella, J. R., Lustig, C., Bucher, L. A., Jha, A. P., & Doraiswamy, P. M. (2002). Prefrontal activation patterns in subjects at risk for Alzheimer disease. *Am J Geriatr Psychiatry, 10*(1), 112-113.

Petrides, M., & Milner, B. (1982). Deficits on subject-ordered tasks after frontal- and temporal-lobe lesions in man. *Neuropsychologia, 20*(3), 249-262.

Petronis, A. (2006). Epigenetics and twins:Three variations on the theme. *Trends Genet, 22*(7), 347-350.

Petrovitch, H., White, L. R., Izmirilian, G., Ross, G. W., Havlik, R. J., Markesbery, W., et al. (2000). Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: The HAAS. Honolulu-Asia aging Study. *Neurobiol Aging, 21*(1), 57-62.

Pievani, M., Rasser, P. E., Galluzzi, S., Benussi, L., Ghidoni, R., Sabattoli, F., et al. (2009). Mapping the effect of APOE epsilon 4 on gray matter loss in Alzheimer's disease in vivo. [Article]. *Neuroimage, 45*(4), 1090-1098.

Pihlajamaki, M., Tanila, H., Hanninen, T., Kononen, M., Laakso, M., Partanen, K., et al. (2000). Verbal fluency activates the left medial temporal lobe: a functional magnetic resonance imaging study. *Ann Neurol, 47*(4), 470-476.

Pitas, R. E., Boyles, J. K., Lee, S. H., Foss, D., & Mahley, R. W. (1987). Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochim Biophys Acta, 917*(1), 148-161.

Pitas, R. E., Ji, Z. S., Weisgraber, K. H., & Mahley, R. W. (1998). Role of apolipoprotein E in modulating neurite outgrowth: Potential effect of intracellular apolipoprotein E. *Biochem Soc Trans, 26*(2), 257-262.

Plassman, B. L., Havlik, R. J., Steffens, D. C., Helms, M. J., Newman, T. N., Drosdick, D., et al. (2000). Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology, 55*(8), 1158-1166.

Plassman, B. L., Welsh-Bohmer, K. A., Bigler, E. D., Johnson, S. C., Anderson, C. V., Helms, M. J., et al. (1997). Apolipoprotein E epsilon 4 allele and hippocampal volume in twins with normal cognition. *Neurology, 48*(4), 985-989.

Plomin, R., & Spinath, F. M. (2002). Genetics and general cognitive ability *Trends Cogn Sci, 6*(4), 169-176.

Poirier, J. (2000). Apolipoprotein E and Alzheimer's disease: A role in amyloid catabolism. *Ann N Y Acad Sci, 924*, 81-90.

Polvikoski, T., Sulkava, R., Haltia, M., Kainulainen, K., Vuorio, A., Verkkoniemi, A., et al. (1995). Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N Engl J Med, 333*(19), 1242-1247.

Price, D. L., & Sisodia, S. S. (1998). Mutant genes in familial Alzheimer's disease and transgenic models. *Annu Rev Neurosci, 21*, 479-505.

Prince, J. A., Feuk, L., Sawyer, S. L., Gottfries, J., Ricksten, A., Nagga, K., et al. (2001). Lack of replication of association findings in complex disease: An analysis of 15 polymorphisms in prior candidate genes for sporadic Alzheimer's disease. *Eur J Hum Genet, 9*(6), 437-444.

Qiu, Z., Crutcher, K. A., Hyman, B. T., & Rebeck, G. W. (2003). ApoE isoforms affect neuronal N-methyl-D-aspartate calcium responses and toxicity via receptor-mediated processes. *Neuroscience, 122*(2), 291-303.

Quinlan, P. T. (1992). *Oxford psycholinguistic database.* Oxoford: Oxford University Press.

Raber, J. (2009). The impact of ApoE on cognition and behavior. *Alzheimer's and Dementia, 5*(4, Supplement 1), P168-P168.

Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R. E., et al. (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: Increased susceptibility of females. *Proc Natl Acad Sci U S A, 95*(18), 10914-10919.

Raber, J., Wong, D., Yu, G. Q., Buttini, M., Mahley, R. W., Pitas, R. E., et al. (2000). Apolipoprotein E and cognitive performance. *Nature, 404*(6776), 352-354.

Rapp, M. A., & Reischies, F. M. (2005). Attention and executive control predict Alzheimer disease in late life: Results from the Berlin Aging Study (BASE). *Am J Geriatr Psychiatry, 13*(2), 134-141.

Rask-Nissila, L., Jokinen, E., Viikari, J., Tammi, A., Ronnemaa, T., Marniemi, J., et al. (2002). Impact of dietary intervention, sex, and apolipoprotein E phenotype on tracking of serum lipids and apolipoproteins in 1- to 5-year-old children: The Special Turku Coronary Risk Factor Intervention Project (STRIP). *Arterioscler Thromb Vasc Biol, 22*(3), 492-498.

Ravaja, N., Raikkonen, K., Lyytinen, H., Lehtimaki, T., & Keltikangas-Jarvinen, L. (1997). Apolipoprotein E phenotypes and cardiovascular responses to experimentally induced mental stress in adolescent boys. *J Behav Med, 20*(6), 571-587.

Razin, A. (1998). CpG methylation, chromatin structure and gene silencing-a three-way connection. *Embo J, 17*(17), 4905-4908.

Razin, A., & Riggs, A. D. (1980). DNA methylation and gene function. *Science, 210*(4470), 604-610.

Razin, A., Szyf, M., Kafri, T., Roll, M., Giloh, H., Scarpa, S., et al. (1986). Replacement of 5-methylcytosine by cytosine: A possible mechanism for transient DNA demethylation during differentiation. *Proc Natl Acad Sci U S A, 83*(9), 2827-2831.

Rebeck, G. W., Reiter, J. S., Strickland, D. K., & Hyman, B. T. (1993). Apolipoprotein E in sporadic Alzheimer's disease: Allelic variation and receptor interactions. *Neuron, 11*(4), 575-580.

Reiman, E. M., Caselli, R. J., Chen, K., Alexander, G. E., Bandy, D., & Frost, J. (2001). Declining brain activity in cognitively normal apolipoprotein E epsilon 4 heterozygotes: A foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease. *Proc Natl Acad Sci U S A, 98*(6), 3334-3339.

Reiman, E. M., Caselli, R. J., Yun, L. S., Chen, K., Bandy, D., Minoshima, S., et al. (1996). Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N Engl J Med, 334*(12), 752-758.

Reiman, E. M., Chen, K., Alexander, G. E., Caselli, R. J., Bandy, D., Osborne, D., et al. (2005). Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. *Proc Natl Acad Sci U S A, 102*(23), 8299-8302.

Reiman, E. M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., et al. (2009). Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A, 106*(16), 6820-6825.

Reiman, E. M., Chen, K. W., Alexander, G. E., Caselli, R. J., Bandy, D., Osborne, D., et al. (2004). Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proceedings of the National Academy of Sciences of the United States of America, 101*(1), 284-289.

Reiman, E. M., Uecker, A., Caselli, R. J., Lewis, S., Bandy, D., de Leon, M. J., et al. (1998a). Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann Neurol, 44*(2), 288-291.

Reiman, E. M., Uecker, A., Caselli, R. J., Lewis, S., Bandy, D., de Leon, M. J., et al. (1998b). Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Annals of Neurology, 44*(2), 288-291.

Reiman, E. M., Webster, J. A., Myers, A. J., Hardy, J., Dunckley, T., Zismann, V. L., et al. (2007). GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. *Neuron, 54*(5), 713-720.

Reinvang, I., Winjevoll, I. L., Rootwelt, H., & Espeseth, T. (2010). Working memory deficits in healthy APOE epsilon 4 carriers. *Neuropsychologia, 48*(2), 566-573.

Reitan, R. M. (1955). The relation of the trail making test to organic brain damage. *J Consult Psychol, 19*(5), 393-394.

Reitz, C., & Mayeux, R. (2009). Endophenotypes in normal brain morphology and Alzheimer's disease: A review. *Neuroscience, 164*(1), 174-190.

Reynolds, C. A., Prince, J. A., Feuk, L., Brookes, A. J., Gatz, M., & Pedersen, N. L. (2006). Longitudinal memory performance during normal aging: Twin association models of APOE and other Alzheimer candidate genes. *Behav Genet, 36*(2), 185-194.

Richey, P. L., Siedlak, S. L., Smith, M. A., & Perry, G. (1995). Apolipoprotein E interaction with the neurofibrillary tangles and senile plaques in Alzheimer disease: Implications for disease pathogenesis. *Biochem Biophys Res Commun, 208*(2), 657-663.

Ridha, B. H., Barnes, J., Bartlett, J. W., Godbolt, A., Pepple, T., Rossor, M. N., et al. (2006). Tracking atrophy progression in familial Alzheimer's disease: A serial MRI study. *Lancet Neurol, 5*(10), 828-834.

Riley, K. P., Snowdon, D. A., Saunders, A. M., Roses, A. D., Mortimer, J. A., & Nanayakkara, N. (2000). Cognitive function and apolipoprotein E in very old adults: Findings from the Nun Study. *J Gerontol B Psychol Sci Soc Sci, 55*(2), S69-75.

Risacher, S. L., Saykin, A. J., West, J. D., Shen, L., Firpi, H. A., McDonald, B. C., et al. (2009). Baseline MRI predictors of conversion from MCI to probable AD in the ADNI cohort. *Curr Alzheimer Res, 6*(4), 347-361.

Ritchie, K., Artero, S., & Touchon, J. (2001). Classification criteria for mild cognitive impairment - A population-based validation study. *Neurology, 56*(1), 37-42.

Ritchie, K., & Kildea, D. (1995). Is senile dementia "age-related" or "ageing-related"? evidence from meta-analysis of dementia prevalence in the oldest old. *The Lancet*, *346*(8980), 931-934.

Rocca, W. A., Bonaiuto, S., Lippi, A., Luciani, P., Turtu, F., Cavarzeran, F., et al. (1990). Prevalence of clinically diagnosed Alzheimer's disease and other dementing disorders: A door-to-door survey in Appignano, Macerata Province, Italy. *Neurology, 40*(4), 626-631.

Rogaeva, E. A., Fafel, K. C., Song, Y. Q., Medeiros, H., Sato, C., Liang, Y., et al. (2001). Screening for PS1 mutations in a referral-based series of AD cases: 21 novel mutations. *Neurology*, *57*(4), 621-625.

Roher, A. E., Ball, M. J., Bhave, S. V., & Wakade, A. R. (1991). Beta-amyloid from Alzheimer disease brains inhibits sprouting and survival of sympathetic neurons. *Biochem Biophys Res Commun, 174*(2), 572-579.

Roman, G. C., Tatemichi, T. K., Erkinjuntti, T., Cummings, J. L., Masdeu, J. C., Garcia, J. H., et al. (1993). Vascular dementia: Diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology, 43*(2), 250-260.

Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., et al. (2008). Heart disease and stroke statistics--2008 update: A report from the American

Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation, 117*(4), e25-146.

Rosen, V. M., Bergeson, J. L., Putnam, K., Harwell, A., & Sunderland, T. (2002). Working memory and apolipoprotein E: What's the connection? *Neuropsychologia*, *40*(13), 2226-2233.

Roses, A. D. (1996). Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu Rev Med, 47*, 387-400.

Ruitenberg, A., van Swieten, J. C., Witteman, J. C., Mehta, K. M., van Duijn, C. M., Hofman, A., et al. (2002). Alcohol consumption and risk of dementia: The Rotterdam Study. *Lancet*, *359*(9303), 281-286.

Salloway, S., Gur, T., Berzin, T., Tavares, R., Zipser, B., Correia, S., et al. (2002). Effect of APOE genotype on microvascular basement membrane in Alzheimer's disease. *J Neurol Sci, 203-204*, 183-187.

Salo, A., Ylikoski, R., Verkkoniemi, A., Polvikoski, T., Juva, K., Rastas, S., et al. (2001). Does apolipoprotein E influence learning and memory in the nondemented oldest old? *Int Psychogeriatr*, *13*(4), 451-459.

Saunders, A. M., Strittmatter, W. J., Schmechel, D., George-Hyslop, P. H., Pericak-Vance, M. A., Joo, S. H., et al. (1993). Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology, 43*(8), 1467-1472.

Saunders, A. M., Trowers, M. K., Shimkets, R. A., Blakemore, S., Crowther, D. J., Mansfield, T. A., et al. (2000). The role of apolipoprotein E in Alzheimer's disease: Pharmacogenomic target selection. *Biochim Biophys Acta, 1502*(1), 85-94.

Saxton, J., Lopez, O. L., Ratcliff, G., Dulberg, C., Fried, L. P., Carlson, M. C., et al. (2004). Preclinical Alzheimer disease: Neuropsychological test performance 1.5 to 8 years prior to onset. *Neurology, 63*(12), 2341-2347.

Scarmeas, N., Anderson, K. E., Hilton, J., Park, A., Habeck, C., Flynn, J., et al. (2004b). APOE-dependent PET patterns of brain activation in Alzheimer disease. *Neurology, 63*(5), 913-915.

Scarmeas, N., Brandt, J., Albert, M., Devanand, D. P., Marder, K., Bell, K., et al. (2002). Association between the APOE genotype and psychopathologic symptoms in Alzheimer's disease. *Neurology, 58*(8), 1182-1188.

Scarmeas, N., Habeck, C., Anderson, K. E., Hilton, J., Devanand, D. P., Pelton, G. H., et al. (2004a). Altered PET functional brain responses in cognitively intact elderly persons at risk for Alzheimer disease (carriers of the epsilon4 allele). *Am J Geriatr Psychiatry, 12*(6), 596-605.

Scarmeas, N., Habeck, C. G., Hilton, J., Anderson, K. E., Flynn, J., Park, A., et al. (2005a). APOE related alterations in cerebral activation even at college age. *J Neurol Neurosurg Psychiatry, 76*(10), 1440-1444.

Scarmeas, N., Habeck, C. G., Stern, Y., & Anderson, K. E. (2003). APOE genotype and cerebral blood flow in healthy young individuals. *Jama, 290*(12), 1581-1582.

Scarmeas, N., Levy, G., Tang, M. X., Manly, J., & Stern, Y. (2001). Influence of leisure activity on the incidence of Alzheimer's disease. *Neurology, 57*(12), 2236-2242.

Scarmeas, N., Luchsinger, J. A., Schupf, N., Brickman, A. M., Cosentino, S., Tang, M. X., et al. (2009). Physical activity, diet, and risk of Alzheimer disease. *JAMA, 302*(6), 627-637.

Scarmeas, N., & Stern, Y. (2005). fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology, 65*(9), 1514-1515; author reply 1514-1515.

Scarmeas, N., & Stern, Y. (2006). Imaging studies and APOE genotype in persons at risk for Alzheimer's disease. *Curr Psychiatry Rep, 8*(1), 11-17.

Scarpa, S., Cavallaro, R. A., D'Anselmi, F., & Fuso, A. (2006). Gene silencing through methylation: An epigenetic intervention on Alzheimer disease. *J Alzheimers Dis, 9*(4), 407-414.

Scarpa, S., Fuso, A., D'Anselmi, F., & Cavallaro, R. A. (2003). Presenilin 1 gene silencing by S-adenosylmethionine: A treatment for Alzheimer disease? *FEBS Lett, 541*(1-3), 145-148.

Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B. J., Hulette, C. M., Joo, S. H., et al. (1993). Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A, 90*(20), 9649-9653.

Schmidt-Wilcke, T., Poljansky, S., Hierlmeier, S., Hausner, J., & Ibach, B. (2009). Memory performance correlates with gray matter density in the ento-/perirhinal cortex and posterior hippocampus in patients with mild cognitive impairment and healthy controls A voxel based morphometry study. *Neuroimage, 47*(4), 1914-1920.

Schoonenboom, S. N., Visser, P. J., Mulder, C., Lindeboom, J., Van Elk, E. J., Van Kamp, G. J., et al. (2005). Biomarker profiles and their relation to clinical variables in mild cognitive impairment. *Neurocase, 11*(1), 8-13.

Schultz, M. R., Lyons, M. J., Franz, C. E., Grant, M. D., Boake, C., Jacobson, K. C., et al. (2008). Apolipoprotein E genotype and memory in the sixth decade of life. *Neurology, 70*(19 Pt 2), 1771-1777.

Schupf, N., Kapell, D., Nightingale, B., Lee, J. H., Mohlenhoff, J., Bewley, S., et al. (2001). Specificity of the fivefold increase in AD in mothers of adults with Down syndrome. *Neurology, 57*(6), 979-984.

Scott, W. K., Grubber, J. M., Conneally, P. M., Small, G. W., Hulette, C. M., Rosenberg, C. K., et al. (2000). Fine mapping of the chromosome 12 late-onset Alzheimer disease locus:Potential genetic and phenotypic heterogeneity. *Am J Hum Genet, 66*(3), 922-932.

Seidenberg, M., Guidotti, L., Nielson, K. A., Woodard, J. L., Durgerian, S., Antuono, P., et al. (2009). Semantic memory activation in individuals at risk for developing Alzheimer disease. *Neurology, 73*(8), 612-620.

Selkoe, D. J. (1994). Alzheimer's disease: A central role for amyloid. *J Neuropathol Exp Neurol, 53*(5), 438-447.

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science, 298*(5594), 789-791.

Semenza, C., Borgo, F., Mondini, S., Pasini, M., & Sgaramella, T. (2000). Proper names in the early stages of Alzheimer's disease. *Brain Cogn, 43*(1-3), 384-387.

Sereno, S. C., O'Donnell, Patrick J. (2009). Participant and Word Gender in Age of Acquisition Effects: The Role of Gender Socialization *Sex Roles, 61*, 510-518

Seshadri, S., & Wolf, P. A. (2007). Lifetime risk of stroke and dementia: Current concepts, and estimates from the Framingham Study. *The Lancet Neurology, 6*(12), 1106-1114.

Shaw, P., Lerch, J. P., Pruessner, J. C., Taylor, K. N., Rose, A. B., Greenstein, D., et al. (2007). Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: An observational study. *Lancet Neurology, 6*(6), 494-500.

Silveri, M. C., Cappa, A., Mariotti, P., & Puopolo, M. (2002). Naming in patients with Alzheimer's disease: Influence of age of acquisition and categorical effects. *J Clin Exp Neuropsychol, 24*(6), 755-764.

Siu, L. L., Chan, J. K., Wong, K. F., Choy, C., & Kwong, Y. L. (2003). Aberrant promoter CpG methylation as a molecular marker for disease monitoring in natural killer cell lymphomas. *Br J Haematol, 122*(1), 70-77.

Slooter, A. J., Breteler, M. B., Ott, A., Van Broeckhoven, C., & van Duijn, C. M. (1996). APOE genotyping in differential diagnosis of Alzheimer's disease. *Lancet, 348*(9023), 334.

Slooter, A. J., Cruts, M., Kalmijn, S., Hofman, A., Breteler, M. M., Van Broeckhoven, C., et al. (1998). Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: The Rotterdam Study. *Arch Neurol, 55*(7), 964-968.

Small, B. J., Herlitz, A., Fratiglioni, L., Almkvist, O., & Backman, L. (1997b). Cognitive predictors of incident Alzheimer's disease: A prospective longitudinal study. *Neuropsychology, 11*(3), 413-420.

Small, B. J., Rosnick, C. B., Fratiglioni, L., & Backman, L. (2004). Apolipoprotein E and cognitive performance: A meta-analysis. *Psychol Aging, 19*(4), 592-600.

Small, G. W., Ercoli, L. M., Silverman, D. H., Huang, S. C., Komo, S., Bookheimer, S. Y., et al. (2000). Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A, 97*(11), 6037-6042.

Small, G. W., La Rue, A., Komo, S., Kaplan, A., & Mandelkern, M. A. (1997). Mnemonics usage and cognitive decline in age-associated memory impairment. *Int Psychogeriatr, 9*(1), 47-56.

Small, G. W., Mazziotta, J. C., Collins, M. T., Baxter, L. R., Phelps, M. E., Mandelkern, M. A., et al. (1995). Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. *Jama, 273*(12), 942-947.

Small, G. W., Siddarth, P., Burggren, A. C., Kepe, V., Ercoli, L. M., Miller, K. J., et al. (2009). Influence of cognitive status, age, and APOE-4 genetic risk on brain FDDNP positron-emission tomography imaging in persons without dementia. *Arch Gen Psychiatry, 66*(1), 81-87.

Smith, C. D., Andersen, A. H., Kryscio, R. J., Schmitt, F. A., Kindy, M. S., Blonder, L. X., et al. (1999). Altered brain activation in cognitively intact individuals at high risk for Alzheimer's disease. *Neurology, 53*(7), 1391-1396.

Smith, C. D., Andersen, A. H., Kryscio, R. J., Schmitt, F. A., Kindy, M. S., Blonder, L. X., et al. (2002). Women at risk for AD show increased parietal activation during a fluency task. *Neurology, 58*(8), 1197-1202.

Smith, C. D., Kryscio, R. J., Schmitt, F. A., Lovell, M. A., Blonder, L. X., Rayens, W. S., et al. (2005). Longitudinal functional alterations in asymptomatic women at risk for Alzheimer's disease. *J Neuroimaging, 15*(3), 271-277.

Smith, E. E., Egorova, S., Blacker, D., Killiany, R. J., Muzikansky, A., Dickerson, B. C., et al. (2008). Magnetic resonance imaging white matter hyperintensities and brain volume in the prediction of mild cognitive impairment and dementia. *Arch Neurol, 65*(1), 94-100.

Smith, J. D. (2002). Apolipoproteins and aging: Emerging mechanisms. *Ageing Res Rev, 1*(3), 345-365.

Snowdon, D. A., Greiner, L. H., & Markesbery, W. R. (2000). Linguistic ability in early life and the neuropathology of Alzheimer's disease and cerebrovascular disease.Findings from the Nun Study. *Ann N Y Acad Sci, 903*, 34-38.

Snowdon, D. A., Kemper, S. J., Mortimer, J. A., Greiner, L. H., Wekstein, D. R., & Markesbery, W. R. (1996). Linguistic ability in early life and cognitive function and Alzheimer's disease in late life. Findings from the Nun Study. *Jama, 275*(7), 528-532.

Soininen, H., Partanen, K., Pitkanen, A., Hallikainen, M., Hanninen, T., Helisalmi, S., et al. (1995). Decreased hippocampal volume asymmetry on MRIs in nondemented elderly subjects carrying the apolipoprotein E epsilon 4 allele. *Neurology, 45*(2), 391-392.

Soininen, H. S., & Riekkinen, P. J., Sr. (1996). Apolipoprotein E, memory and Alzheimer's disease. *Trends Neurosci, 19*(6), 224-228.

Song, Y., Stampfer, M. J., & Liu, S. (2004). Meta-analysis: Apolipoprotein E genotypes and risk for coronary heart disease. *Ann Intern Med, 141*(2), 137-147.

Sorg, C., Riedl, V., Muhlau, M., Calhoun, V. D., Eichele, T., Laer, L., et al. (2007). Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. *Proc Natl Acad Sci U S A, 104*(47), 18760-18765.

Sparks, D. L., Scheff, S. W., Liu, H., Landers, T., Danner, F., Coyne, C. M., et al. (1996). Increased density of senile plaques (SP), but not neurofibrillary tangles (NFT), in non-demented individuals with the apolipoprotein E4 allele: comparison to confirmed Alzheimer's disease patients. *J Neurol Sci, 138*(1-2), 97-104.

Sperling, R. A., Bates, J. F., Chua, E. F., Cocchiarella, A. J., Rentz, D. M., Rosen, B. R., et al. (2003). fMRI studies of associative encoding in young and elderly controls and mild Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 74*(1), 44-50.

Srinivasan, S. R., Ehnholm, C., Elkasabany, A., & Berenson, G. S. (2001). Apolipoprotein E polymorphism modulates the association between obesity and dyslipidemias during young adulthood: The Bogalusa Heart Study. *Metabolism, 50*(6), 696-702.

St George-Hyslop, P. H. (2000). Genetic factors in the genesis of Alzheimer's disease. *Ann N Y Acad Sci, 924*, 1-7.

Standish, T. I., Molloy, D. W., Cunje, A., & Lewis, D. L. (2007). Do the ABCS 135 short cognitive screen and its subtests discriminate between normal cognition, mild cognitive impairment and dementia? *Int J Geriatr Psychiatry, 22*(3), 189-194.

Stein, J., Luppa, M., Brahler, E., Konig, H. H., & Riedel-Heller, S. G. (2010). The assessment of changes in cognitive functioning: Reliable change indices for neuropsychological instruments in the elderly a systematic review. *Dement Geriatr Cogn Disord, 29*(3), 275-286.

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *J Int Neuropsychol Soc, 8*(3), 448-460.

Stern, Y., Gurland, B., Tatemichi, T. K., Tang, M. X., Wilder, D., & Mayeux, R. (1994). Influence of education and occupation on the incidence of Alzheimer's disease. *JAMA, 271*(13), 1004-1010.

Stern, Y., Tang, M. X., Albert, M. S., Brandt, J., Jacobs, D. M., Bell, K., et al. (1997). Predicting time to nursing home care and death in individuals with Alzheimer disease. *JAMA, 277*(10), 806-812.

Stevens, T., Livingston, G., Kitchen, G., Manela, M., Walker, Z., & Katona, C. (2002a). Islington study of dementia subtypes in the community. *Br J Psychiatry, 180*, 270-276.

Stone, D. J., Rozovsky, I., Morgan, T. E., Anderson, C. P., & Finch, C. E. (1998). Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. *J Neurosci, 18*(9), 3180-3185.

Stoub, T. R., deToledo-Morrell, L., Stebbins, G. T., Leurgans, S., Bennett, D. A., & Shah, R. C. (2006). Hippocampal disconnection contributes to memory dysfunction in individuals at risk for Alzheimer's disease. *Proc Natl Acad Sci U S A, 103*(26), 10041-10045.

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., et al. (1993). Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A, 90*(5), 1977-1981.

Sunderland, T., Mirza, N., Putnam, K. T., Linker, G., Bhupali, D., Durham, R., et al. (2004). Cerebrospinal fluid beta-amyloid1-42 and tau in control subjects at risk for Alzheimer's disease: The effect of APOE epsilon4 allele. *Biol Psychiatry, 56*(9), 670-676.

Tabaton, M., Mandybur, T. I., Perry, G., Onorato, M., Autilio-Gambetti, L., & Gambetti, P. (1989). The widespread alteration of neurites in Alzheimer's disease may be unrelated to amyloid deposition. *Ann Neurol, 26*(6), 771-778.

Tabert, M. H., Manly, J. J., Liu, X., Pelton, G. H., Rosenblum, S., Jacobs, M., et al. (2006). Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment. *Arch Gen Psychiatry, 63*(8), 916-924.

Tammi, A., Ronnemaa, T., Rask-Nissila, L., Miettinen, T. A., Gylling, H., Valsta, L., et al. (2001). Apolipoprotein E phenotype regulates cholesterol absorption in healthy 13-month-old children The STRIP Study. *Pediatr Res, 50*(6), 688-691.

Tanaka, S., Kawamata, J., Shimohama, S., Akaki, H., Akiguchi, I., Kimura, J., et al. (1998). Inferior temporal lobe atrophy and APOE genotypes in Alzheimer's disease: X-ray computed tomography, magnetic resonance imaging and Xe-133 SPECT studies. *Dement Geriatr Cogn Disord, 9*(2), 90-98.

Tang, M. X., Cross, P., Andrews, H., Jacobs, D. M., Small, S., Bell, K., et al. (2001). Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. *Neurology, 56*(1), 49-56.

Tang, M. X., Stern, Y., Marder, K., Bell, K., Gurland, B., Lantigua, R., et al. (1998). The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. *JAMA, 279*(10), 751-755.

Tapiola, T., Pennanen, C., Tapiola, M., Tervo, S., Kivipelto, M., Hanninen, T., et al. (2008). MRI of hippocampus and entorhinal cortex in mild cognitive impairment: A follow-up study. *Neurobiol Aging, 29*(1), 31-38.

Teter, B., Xu, P. T., Gilbert, J. R., Roses, A. D., Galasko, D., & Cole, G. M. (1999). Human apolipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture. *J Neurochem, 73*(6), 2613-2616.

Thaker, U., McDonagh, A. M., Iwatsubo, T., Lendon, C. L., Pickering-Brown, S. M., & Mann, D. M. (2003). Tau load is associated with apolipoprotein E genotype and the amount of amyloid beta protein, Abeta40, in sporadic and familial Alzheimer's disease. *Neuropathol Appl Neurobiol, 29*(1), 35-44.

Thambisetty, M., Beason-Held, L., An, Y., Kraut, M. A., & Resnick, S. M. (2010). APOE epsilon4 genotype and longitudinal changes in cerebral blood flow in normal aging. *Arch Neurol, 67*(1), 93-98.

Thomann, P. A., Roth, A. S., Dos Santos, V., Toro, P., Essig, M., & Schroder, J. (2008). Apolipoprotein E Polymorphism and Brain Morphology in Mild Cognitive Impairment. *Dementia and Geriatric Cognitive Disorders, 26*(4), 300-305.

Thompson, P., Cannon, T. D., & Toga, A. W. (2002). Mapping genetic influences on human brain structure. *Ann Med, 34*(7-8), 523-536.

Thompson, P. M., Cannon, T. D., Narr, K. L., van Erp, T., Poutanen, V. P., Huttunen, M., et al. (2001). Genetic influences on brain structure. *Nat Neurosci, 4*(12), 1253-1258.

Thompson, P. M., Hayashi, K. M., Dutton, R. A., Chiang, M. C., Leow, A. D., Sowell, E. R., et al. (2007). Tracking Alzheimer's disease. *Ann N Y Acad Sci, 1097*, 183-214.

Tiraboschi, P., Hansen, L. A., Masliah, E., Alford, M., Thal, L. J., & Corey-Bloom, J. (2004). Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. *Neurology, 62*(11), 1977-1983.

Tirado, V., Munoz, C., Aguirre, C., Pineda, D. A., & Lopera, F. (2004). Performance of carriers and non-carriers of the E280A mutation for familial Alzheimer's disease in a naming test. *Rev Neurol, 39*(4), 322-326.

Trivedi, M. A., Schmitz, T. W., Ries, M. L., Torgerson, B. M., Sager, M. A., Hermann, B. P., et al. (2006). Reduced hippocampal activation during episodic encoding in middle-aged individuals at genetic risk of Alzheimer's Disease: A cross-sectional study. *Bmc Medicine, 4*, 14.

Trommer, B. L., Shah, C., Yun, S. H., Gamkrelidze, G., Pasternak, E. S., Stine, W. B., et al. (2005). ApoE isoform-specific effects on LTP: Blockade by oligomeric amyloid-beta1-42. *Neurobiol Dis, 18*(1), 75-82.

Troyer, A. K. (2000). Normative data for clustering and switching on verbal fluency tasks. *J Clin Exp Neuropsychol, 22*(3), 370-378.

Tsankova, N., Renthal, W., Kumar, A., & Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci, 8*(5), 355-367.

Tupler, L. A., Krishnan, K. R., Greenberg, D. L., Marcovina, S. M., Payne, M. E., MacFall, J. R., et al. (2007). Predicting memory decline in normal elderly: Genetics, MRI, and cognitive reserve. *Neurobiol Aging, 28*(11), 1644-1656.

Turic, D., Fisher, P. J., Plomin, R., & Owen, M. J. (2001). No association between apolipoprotein E polymorphisms and general cognitive ability in children. *Neurosci Lett, 299*(1-2), 97-100.

Twamley, E. W., Ropacki, S. A., & Bondi, M. W. (2006). Neuropsychological and neuroimaging changes in preclinical Alzheimer's disease. *J Int Neuropsychol Soc, 12*(5), 707-735.

Ulrey, C. L., Liu, L., Andrews, L. G., & Tollefsbol, T. O. (2005). The impact of metabolism on DNA methylation. *Hum Mol Genet, 14 Spec No 1*, R139-147.

Urdinguio, R. G., Sanchez-Mut, J. V., & Esteller, M. (2009). Epigenetic mechanisms in neurological diseases: Genes, syndromes, and therapies. *Lancet Neurology, 8*(11), 1056-1072.

Uryu, K., Laurer, H., McIntosh, T., Pratico, D., Martinez, D., Leight, S., et al. (2002). Repetitive mild brain trauma accelerates Abeta deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. *J Neurosci, 22*(2), 446-454.

Utermann, G., Pruin, N., & Steinmetz, A. (1979). Polymorphism of apolipoprotein E. III. Effect of a single polymorphic gene locus on plasma lipid levels in man. *Clin Genet, 15*(1), 63-72.

Valastro, B., Ghribi, O., Poirier, J., Krzywkowski, P., & Massicotte, G. (2001). AMPA receptor regulation and LTP in the hippocampus of young and aged apolipoprotein E-deficient mice. *Neurobiol Aging, 22*(1), 9-15.

van Bockxmeer, F. M., & Mamotte, C. D. (1992). Apolipoprotein epsilon 4 homozygosity in young men with coronary heart disease. *Lancet, 340*(8824), 879-880.

van de Pol, L. A., van der Flier, W. M., Korf, E. S., Fox, N. C., Barkhof, F., & Scheltens, P. (2007). Baseline predictors of rates of hippocampal atrophy in mild cognitive impairment. *Neurology, 69*(15), 1491-1497.

Van Gassen, G., & Annaert, W. (2003). Amyloid, presenilins, and Alzheimer's disease. *Neuroscientist, 9*(2), 117-126.

Van Speybroeck, L. (2002). From epigenesis to epigenetics: The case of C. H. Waddington. *Ann N Y Acad Sci, 981*, 61-81.

van Vliet, J., Oates, N. A., & Whitelaw, E. (2007). Epigenetic mechanisms in the context of complex diseases. *Cell Mol Life Sci, 64*(12), 1531-1538.

Vandenberghe, R., Price, C., Wise, R., Josephs, O., & Frackowiak, R. S. (1996). Functional anatomy of a common semantic system for words and pictures. *Nature, 383*(6597), 254-256.

Vandenbulcke, M., Peeters, R., Dupont, P., Van Hecke, P., & Vandenberghe, R. (2007). Word reading and posterior temporal dysfunction in amnestic mild cognitive impairment. *Cereb Cortex, 17*(3), 542-551.

Venneri, A., Forbes-Mckay, K. E., & Shanks, M. F. (2005). Impoverishment of spontaneous language and the prediction of Alzheimer's disease. *Brain, 128*(Pt 4), E27.

Venneri, A., McGeown, W. J., Hietanen, H. M., Guerrini, C., Ellis, A. W., & Shanks, M. F. (2008). The anatomical bases of semantic retrieval deficits in early Alzheimer's disease. *Neuropsychologia, 46*(2), 497-510.

Venneri, A., Tumbull, O., & Della Sala, S. (1996). The taxonomic perspective: The neuropsychological diagnosis of dementia. *Revue Europeenne de Psychologie Aplique, 46*, 179-190.

Viereck, C., Tucker, R. P., & Matus, A. (1989). The adult rat olfactory system expresses microtubule-associated proteins found in the developing brain. *J Neurosci, 9*(10), 3547-3557.

Visser, P. J., Scheltens, P., Verhey, F. R., Schmand, B., Launer, L. J., Jolles, J., et al. (1999a). Medial temporal lobe atrophy and memory dysfunction as predictors for dementia in subjects with mild cognitive impairment. *J Neurol, 246*(6), 477-485.

Vogel, A., Gade, A., Stokholm, J., & Waldemar, G. (2005). Semantic memory impairment in the earliest phases of Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders, 19*(2-3), 75-81.

Wang, C., Stebbins, G. T., Medina, D. A., Shah, R. C., Bammer, R., Moseley, M. E., et al. (2010). Atrophy and dysfunction of parahippocampal white matter in mild Alzheimer's disease. *Neurobiol Aging, In Press, Corrected Proof*.

Wang, C., Wilson, W. A., Moore, S. D., Mace, B. E., Maeda, N., Schmechel, D. E., et al. (2005). Human apoE4-targeted replacement mice display synaptic deficits in the absence of neuropathology. *Neurobiol Dis, 18*(2), 390-398.

Wang, S. C., Oelze, B., & Schumacher, A. (2008). Age-specific epigenetic drift in late-onset Alzheimer's disease. *PLoS One, 3*(7), e2698.

Wang, W., Wu, S., Cheng, X., Dai, H., Ross, K., Du, X., et al. (2000). Prevalence of Alzheimer's disease and other dementing disorders in an urban community of Beijing, China. *Neuroepidemiology, 19*(4), 194-200.

Waring, S. C., Rocca, W. A., Petersen, R. C., O'Brien, P. C., Tangalos, E. G., & Kokmen, E. (1999). Postmenopausal estrogen replacement therapy and risk of AD: A population-based study. *Neurology, 52*(5), 965-970.

Waring, S. C., & Rosenberg, R. N. (2008). Genome-wide association studies in Alzheimer disease. *Arch Neurol, 65*(3), 329-334.

Wehling, E., Lundervold, A. J., Standnes, B., Gjerstad, L., & Reinvang, I. (2007). APOE status and its association to learning and memory performance in middle aged and older Norwegians seeking assessment for memory deficits. *Behav Brain Funct, 3*, 57.

Weisgraber, K. H. (1994). Apolipoprotein E structure function relationships *Advances in Protein Chemistry, Vol 45, 45*, 249-302.

Welsh-Bohmer, K. A., Ostbye, T., Sanders, L., Pieper, C. F., Hayden, K. M., Tschanz, J. T., et al. (2009). Neuropsychological performance in advanced age: Influences of demographic factors and Apolipoprotein E: Findings from the Cache County Memory Study. *Clin Neuropsychol, 23*(1), 77-99.

Wetter, S. R., Delis, D. C., Houston, W. S., Jacobson, M. W., Lansing, A., Cobell, K., et al. (2005). Deficits in inhibition and flexibility are associated with the APOE-E4 allele in nondemented older adults. *J Clin Exp Neuropsychol, 27*(8), 943-952.

Whalley, L. J., Starr, J. M., Athawes, R., Hunter, D., Pattie, A., & Deary, I. J. (2000). Childhood mental ability and dementia. *Neurology, 55*(10), 1455-1459.

White, F., Nicoll, J. A., Roses, A. D., & Horsburgh, K. (2001). Impaired neuronal plasticity in transgenic mice expressing human apolipoprotein E4 compared to E3 in a model of entorhinal cortex lesion. *Neurobiol Dis, 8*(4), 611-625.

Whitelaw, N. C., & Whitelaw, E. (2006). How lifetimes shape epigenotype within and across generations. *Hum Mol Genet, 15 Spec No 2*, R131-137.

Whitwell, J. L., Petersen, R. C., Negash, S., Weigand, S. D., Kantarci, K., Ivnik, R. J., et al. (2007). Patterns of atrophy differ among specific subtypes of mild cognitive impairment. *Arch Neurol, 64*(8), 1130-1138.

Whitwell, J. L., Shiung, M. M., Przybelski, S. A., Weigand, S. D., Knopman, D. S., Boeve, B. F., et al. (2008). MRI patterns of atrophy associated with progression to AD in amnestic mild cognitive impairment. *Neurology, 70*(7), 512-520.

Williams, G. C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. *Evolution, 11*(4), 398-411.

Willingham, D. B., Salidis, J., & Gabrieli, J. D. (2002). Direct comparison of neural systems mediating conscious and unconscious skill learning. *J Neurophysiol, 88*(3), 1451-1460.

Wilson, R. S., Schneider, J. A., Barnes, L. L., Beckett, L. A., Aggarwal, N. T., Cochran, E. J., et al. (2002). The apolipoprotein E epsilon 4 allele and decline in different cognitive systems during a 6-year period. *Arch Neurol, 59*(7), 1154-1160.

Winnock, M., Letenneur, L., Jacqmin-Gadda, H., Dallongeville, J., Amouyel, P., & Dartigues, J. F. (2002). Longitudinal analysis of the effect of apolipoprotein E epsilon4 and education on cognitive performance in elderly subjects: The PAQUID study. *J Neurol Neurosurg Psychiatry, 72*(6), 794-797.

Wishart, H. A., Saykin, A. J., McAllister, T. W., Rabin, L. A., McDonald, B. C., Flashman, L. A., et al. (2006a). Regional brain atrophy in cognitively intact adults with a single APOE epsilon 4 allele. *Neurology, 67*(7), 1221-1224.

Wishart, H. A., Saykin, A. J., Rabin, L. A., Santulli, R. B., Flashman, L. A., Guerin, S. J., et al. (2006). Increased brain activation during working memory in cognitively intact adults with the APOE epsilon4 allele. *Am J Psychiatry, 163*(9), 1603-1610.

Wisniewski, T., Castano, E. M., Golabek, A., Vogel, T., & Frangione, B. (1994). Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am J Pathol, 145*(5), 1030-1035.

Wisniewski, T., & Frangione, B. (1992). Apolipoprotein E: A pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci Lett, 135*(2), 235-238.

Wolf, H., Grunwald, M., Kruggel, F., Riedel-Heller, S. G., Angerhofer, S., Hojjatoleslami, A., et al. (2001). Hippocampal volume discriminates between normal cognition: Questionable and mild dementia in the elderly. *Neurobiol Aging, 22*(2), 177-186.

Woodard, J. L., Seidenberg, M., Nielson, K. A., Antuono, P., Guidotti, L., Durgerian, S., et al. (2009). Semantic memory activation in amnestic mild cognitive impairment. *Brain, 132*(Pt 8), 2068-2078.

Wright, R. O., Hu, H., Silverman, E. K., Tsaih, S. W., Schwartz, J., Bellinger, D., et al. (2003). Apolipoprotein E genotype predicts 24-month bayley scales infant development score. *Pediatr Res, 54*(6), 819-825.

Xu, Q., Bernardo, A., Walker, D., Kanegawa, T., Mahley, R. W., & Huang, Y. (2006). Profile and regulation of apolipoprotein E (ApoE) expression in the CNS in mice with targeting of green fluorescent protein gene to the ApoE locus. *J Neurosci, 26*(19), 4985-4994.

Xu, Q., Walker, D., Bernardo, A., Brodbeck, J., Balestra, M. E., & Huang, Y. (2008). Intron-3 retention/splicing controls neuronal expression of apolipoprotein E in the CNS. *J Neurosci, 28*(6), 1452-1459.

Yamaguchi, S., Nakagawa, T., Arai, H., Sasaki, H., Higuchi, S., & Trojanowski, J. Q. (1996). Temporal progression of hippocampal atrophy and apolipoprotein E gene in Alzheimer's disease. *J Am Geriatr Soc, 44*(2), 216-217.

Yankner, B. A. (1996). Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron, 16*(5), 921-932.

Yasuda, M., Mori, E., Kitagaki, H., Yamashita, H., Hirono, N., Shimada, K., et al. (1998). Apolipoprotein E epsilon 4 allele and whole brain atrophy in late-onset Alzheimer's disease. *Am J Psychiatry, 155*(6), 779-784.

Ye, S., Huang, Y., Mullendorff, K., Dong, L., Giedt, G., Meng, E. C., et al. (2005). Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. *Proc Natl Acad Sci U S A, 102*(51), 18700-18705.

Yu, Y. W., Lin, C. H., Chen, S. P., Hong, C. J., & Tsai, S. J. (2000). Intelligence and event-related potentials for young female human volunteer apolipoprotein E epsilon4 and non-epsilon4 carriers. *Neurosci Lett, 294*(3), 179-181.

Zannis, V. I., Breslow, J. L., Utermann, G., Mahley, R. W., Weisgraber, K. H., Havel, R. J., et al. (1982). Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes. *J Lipid Res, 23*(6), 911-914.

Zannis, V. I., Just, P. W., & Breslow, J. L. (1981). Human apolipoprotein E isoprotein subclasses are genetically determined. *Am J Hum Genet, 33*(1), 11-24.

Zheng, H., Xu, H., Uljon, S. N., Gross, R., Hardy, K., Gaynor, J., et al. (2002). Modulation of A beta peptides by estrogen in mouse models. *Journal of Neurochemistry, 80*(1), 191-196.

Zhou, Y., Elkins, P. D., Howell, L. A., Ryan, D. H., & Harris, R. B. (1998). Apolipoprotein-E deficiency results in an altered stress responsiveness in addition to an impaired spatial memory in young mice. *Brain Research, 788*(1-2), 151-159.

Zhu, X., Raina, A. K., Perry, G., & Smith, M. A. (2004). Alzheimer's disease: The two-hit hypothesis. *Lancet Neurology, 3*(4), 219-226.

Zukin, S. (2009). Epigenetics. *Alzheimer's & Dementia, 5*, P146-P147.

APPENDIX A

Table A.1a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to the 18-20 age –category.

Animal	**Mean**	**SD**	**Range min**	**max**
Aardvark #	7.70	3.25	4	15
Adder # ~	6.78	2.94	4	14
Albatross # ~	5.74	4.26	1	18
Alligator # *	6.08	1.48	3	10
Amoeba	13.21	2.34	8	18
Angel fish #	9.38	3.61	4	18
Ant # *	4.25	1.22	3	7
Anteater # ~	7.51	2.39	5	13
Antelope # ~	7.02	2.13	4	11
Ape #	5.41	2.03	3	9
Armadillo #	8.26	2.28	5	12
Ass #	8.02	3.46	4	18
Baboon #	7.29	2.08	4	12
Badger #	5.61	1.86	3	10
Bald eagle #	8.94	3.87	4	18
Bat #	5.34	1.69	3	11
Bear *	4.71	1.74	3	9
Beaver # *	6.70	2.30	3	14
Bee # *	4.38	1.39	3	8
Beetle	5.07	1.87	3	10
Bird	3.62	0.89	3	6
Bison ~	9.65	3.73	5	18
Blackbird	5.45	2.22	3	12
Blue bird #	7.46	3.02	4	18
Blue tit # *	7.72	2.91	4	14
Boa constrictor #	7.97	2.17	4	12
Boar	7.73	2.73	4	15
Brontosaurus # ~	6.73	3.84	4	19
Brown bear #	5.40	2.12	2	10
Buck	9.28	3.92	4	18
Budgerigar #	7.63	4.69	3	19
Buffalo #	7.17	2.75	4	14

Animal	Mean	SD	Range min	max
Bull	6.07	1.90	3	10
Bullfinch	11.02	3.47	5	18
Bullock	8.21	3.88	4	18
Butterfly	4.24	1.53	2	8
Buzzard	10.10	3.82	7	19
Calf	5.73	2.36	3	12
Camel	6.28	2.19	3	11
Canary	6.77	2.43	4	13
Caribou	4.94	5.34	1	18
Carp # ~	8.41	2.67	5	14
Cat	3.49	1.06	2	6
Caterpillar	4.60	1.47	3	8
Cattle	5.42	2.37	3	10
Chaffinch	8.85	2.94	5	14
Chameleon #	7.99	3.05	4	15
Cheetah # ~	6.26	1.81	4	10
Chicken # *	3.99	1.40	2	9
Chimpanzee	6.23	1.80	4	10
Chinchilla #	8.38	2.60	5	15
Chipmunk # ~ *	6.01	2.56	3	16
Clown fish #	11.31	2.99	7	18
Cobra # ~	6.95	2.50	3	14
Cockatiel #	6.89	3.58	3	18
Cocker spaniel	6.42	2.56	3	15
Cockerel	5.84	2.01	3	12
Cockroach	7.67	2.32	4	13
Cod	7.14	2.71	3	13
Condor # ~	10.87	3.08	5	18
Conger eel	10.66	3.28	7	18
Cougar # ~	9.64	3.44	5	18
Cow *	3.48	1.14	1	6
Coyote # ~ *	8.80	3.44	4	17
Cray fish # ~	9.09	4.00	4	18
Crocodile #	4.89	1.81	3	10
Crow	6.23	2.33	3	15
Cuckoo	5.34	3.59	1	18
Deer # *	5.09	1.79	3	11
Dingo	9.00	3.46	4	18
Dog	3.04	0.98	1	5

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Dolphin #	4.80	1.63	2	8
Donkey	4.20	1.52	3	8
Dormouse	6.69	2.48	4	15
Dove #	6.00	2.38	3	11
Dragon #	4.90	1.81	3	10
Dragonfly # *	6.22	2.08	3	11
Duck # *	3.52	1.60	1	8
Duckbill platypus #	8.13	3.62	3	18
Eagle	6.60	2.39	3	14
Earthworm	5.81	3.35	3	17
Earwig *	5.63	2.89	2	13
Eel	7.22	2.65	3	14
Elk	10.31	4.28	4	18
Emu #	8.16	3.09	5	19
Ewe	7.55	3.32	4	14
Ferret	7.14	2.82	3	12
Field mouse	6.58	3.02	3	14
Finch	8.92	3.43	4	14
Fish	3.79	1.07	3	6
Flamingo #	6.17	2.07	3	11
Flea	4.62	2.63	1	13
Fly *	4.54	1.31	3	8
Fowl # ~	8.98	3.50	5	18
Fox # *	4.46	1.14	3	7
Frog # *	4.29	1.51	3	9
Gazelle	8.04	3.40	4	15
Gecko #	5.64	4.10	1	18
Gerbil #	5.91	2.09	2	11
Giant panda #	7.72	3.33	3	16
Gibbon #	10.87	3.87	4	18
Giraffe	5.03	1.68	3	9
Goat	4.74	2.19	3	11
Goldfish	4.43	1.26	3	8
Goose #	5.37	1.72	3	9
Gorilla #	5.23	1.70	3	9
Greyhound	8.23	2.37	6	15
Grizzly bear # *	5.18	2.10	2	10
Groundhog # *	8.76	3.48	4	18
Guinea fowl	11.49	3.12	8	18

Animal	Mean	SD	Range min	Range max
Guinea pig #	5.51	1.74	2	9
Gull	6.50	3.82	3	18
Haddock	7.28	2.86	3	14
Hamster #	4.60	1.53	2	8
Hare	6.08	2.50	4	12
Hawk # ~	7.20	2.39	4	14
Hedgehog	5.09	1.30	3	9
Hen	4.57	2.77	2	12
Heron #	7.74	3.16	4	14
Herring	8.42	2.91	3	15
Hippopotamus #	5.73	1.45	3	9
Hornet	5.24	3.99	1	18
Horse	3.76	1.26	3	7
Horsefly	8.99	3.48	5	18
Hyena #	6.52	3.15	4	16
Iguana # *	8.66	2.85	5	15
Insect #	4.33	1.04	3	7
Invertebrate	8.18	2.19	3	13
Jack rabbit	8.99	4.08	4	18
Jackal # ~	8.98	2.82	4	15
Jackass	10.04	3.95	4	19
Jackdaw	10.23	3.94	4	19
Jaguar #	8.01	2.64	4	15
Kangaroo #	5.70	1.90	3	11
Kid	7.49	4.58	4	18
Kitten	3.96	1.4	2	9
Kiwi	9.40	4.17	5	19
Koala #	6.27	2.58	3	14
Koi carp #	9.70	2.98	5	15
Komodo dragon # ~	9.28	3.68	5	19
Lady bird #	4.32	1.46	2	10
Lamb	4.50	1.85	3	10
Lemur #	8.93	3.27	4	17
Leopard #	6.39	1.72	4	10
Lion # *	4.29	1.27	3	7
Lizard #	5.46	1.88	3	12
Llama	8.66	3.52	4	16
Lobster # ~	7.76	2.11	5	13
Long tailed tit	10.01	3.61	4	18

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Lynx	9.78	3.03	5	16
Mackerel *	9.27	2.69	4	16
Mammal #	6.38	1.88	4	10
Meerkat #	8.05	3.39	4	15
Midge	9.05	2.95	4	18
Mink	10.65	3.38	5	18
Manx	9.03	3.38	4	18
Mole	5.92	2.28	4	13
Mongoose ~	9.18	2.94	5	18
Monkey	4.31	1.57	3	8
Moose # *	7.25	2.13	4	13
Moth	5.89	2.04	3	11
Mouse	4.20	1.33	2	7
Mule	7.96	2.46	6	14
Newt	7.00	3.07	4	14
Nightingale	9.09	3.14	4	17
Octopus #	5.35	1.77	3	9
Orang-utan #	4.81	2.35	1	12
Ostrich #	6.81	2.00	4	12
Otter #	7.18	1.85	4	11
Owl	4.66	1.86	3	9
Ox	8.19	3.59	4	18
Panda #	5.60	2.23	3	10
Panther # ~	6.97	2.56	4	15
Parakeet	8.32	3.85	5	18
Parrot #	5.38	1.34	3	9
Partridge #	7.09	3.15	4	18
Peacock #	5.72	2.01	3	10
Pelican #	6.17	2.36	3	14
Penguin #	4.58	1.95	3	10
Perch	9.67	4.01	4	18
Pheasant	7.83	2.57	4	16
Pig # *	3.61	1.57	1	8
Pigeon	5.05	1.80	2	9
Piglet ~	4.52	1.55	3	9
Pike # ~	8.29	3.02	3	15
Piranha fish # ~	8.08	3.19	4	16
Plaice #	9.50	3.39	4	18
Platypus #	8.42	2.83	5	14

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Polar bear	5.91	2.22	3	12
Pony	4.35	1.76	3	9
Porcupine	8.28	3.03	5	18
Porpoise	9.60	3.95	5	18
Poultry #	7.81	3.62	3	18
Prairie dog #	9.25	3.85	4	18
Puffin #	7.27	2.93	3	18
Puma #	8.27	2.50	4	14
Rabbit # *	3.61	1.39	1	8
Racoon #	7.09	2.82	4	14
Ram	7.53	3.59	3	20
Rat	4.64	1.55	3	9
Raven ~	7.76	2.41	4	13
Reindeer #	3.82	0.98	2	6
Rhesus monkey	8.40	3.86	1	19
Rhinoceros #	5.74	1.34	3	8
Roach	8.95	3.32	5	18
Robin	5.13	1.88	3	10
Rodent	7.40	2.75	3	14
Rook	6.14	4.74	2	18
Rooster # *	4.58	2.05	2	9
Salamander #	9.79	3.54	4	18
Salmon	7.55	2.54	4	14
Sardine	7.78	2.89	4	15
Sea lion #	6.96	2.87	4	14
Seagull	5.22	1.54	3	9
Seahorse #	6.39	2.16	4	14
Seal #	5.61	2.24	3	11
Shark #	5.29	1.66	3	10
Sheep # *	3.95	0.90	3	6
Shrew	9.12	3.56	4	18
Shrimp	7.90	2.88	3	17
Siamese cat	7.02	3.16	4	14
Siberian tiger #	9.28	2.52	5	15
Skate	11.25	3.13	6	18
Skunk #	6.45	2.33	3	12
Skylark # *	10.65	3.73	5	19
Sloth #	9.65	3.36	6	18
Slug #	4.51	1.40	3	8

Animal	Mean	SD	Range min	Range max
Snail	4.58	1.65	3	9
Snake # *	4.59	1.51	3	9
Sole	10.64	2.97	5	18
Sow #	9.34	3.84	4	18
Sparrow #	6.61	2.54	4	12
Spider	3.84	1.16	3	7
Springbok	11.89	3.13	5	18
Squid #	7.16	2.87	4	14
Squirrel # *	4.70	1.59	2	9
Stag	7.94	3.08	4	15
Star fish #	6.15	3.32	3	18
Starling #	8.19	2.96	4	14
Stick insect #	6.59	1.89	4	11
Stickleback #	8.73	3.64	5	18
Stoat	9.51	4.12	5	18
Sturgeon	12.97	2.72	9	19
Swan	4.96	1.68	3	8
Swift	8.82	4.44	5	19
Sword fish	7.84	2.83	4	14
Tarantula #	6.48	2.27	4	13
Thrush # *	9.51	3.32	4	16
Tiger # *	4.58	1.35	3	8
Toad # *	4.92	1.79	3	9
Tortoise	5.53	1.38	3	8
Trout ~	7.48	3.44	3	16
Tuna #	6.89	2.95	3	14
Turkey # *	5.08	2.19	2	10
Turtle # *	5.38	1.45	3	9
Tyrannosaurus # ~ *	5.44	1.99	3	10
Vole	8.00	2.70	4	14
Vulture # ~	7.24	2.68	4	13
Wallaby #	8.05	2.79	5	16
Walrus	7.90	2.29	5	14
Warthog #	7.56	2.44	5	14
Wasp	4.78	1.68	3	10
Water buffalo #	8.53	2.36	4	15
Water rat	8.05	3.69	4	18
Weasel # *	6.81	3.16	4	14
Whale #	5.01	1.83	3	10

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
White tiger #	8.47	2.60	5	15
Wild boar #	7.53	2.66	4	14
Wild cat	7.14	3.12	4	15
Wild dog #	8.38	2.80	4	15
Wildebeest # ~	8.42	3.13	4	15
Wolf	5.26	1.98	3	11
Wombat #	8.63	2.74	4	13
Woodlouse #	5.67	2.02	3	10
Woodpecker	6.71	2.87	4	13
Worm	4.02	1.41	2	7
Wren	8.17	4.12	3	18
Yak #	9.08	3.69	5	18
Yellow tit	9.58	3.22	4	18
Yorkshire terrier *	6.84	3.20	3	15
Zebra # *	5.12	1.57	3	10

Table A.1b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to the 18-20 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	5.74	2.11	3	10
Almond	8.06	3.48	3	19
Apple # *	3.32	1.22	1	8
Apricot #	5.53	3.08	1	14
Aubergine #	9.47	4.27	4	19
Avocado #	9.43	3.84	5	18
Banana # *	3.58	1.32	1	8
Berries	4.54	1.83	1	10
Blackberry	5.55	2.46	2	12
Blackcurrant # *	4.88	2.90	2	15
Blueberry # ~	6.34	3.48	3	17
Bramble ~ *	7.14	4.99	3	18
Cantaloupe melon #	10.78	4.53	4	19
Cherry #	5.07	1.63	2	8
Chestnut	6.91	2.28	4	11
Clementine #	6.12	3.10	3	17
Coconut	6.32	2.26	3	12
Cox apple *	6.13	4.33	3	17
Crab apple	7.87	4.91	4	18
Cranberry #	7.67	3.16	3	16
Cucumber #	4.68	2.23	1	10
Currant	5.14	3.10	1	16
Damson #	9.14	4.38	4	19
Date	7.88	2.65	5	14
Elderberry *	9.31	3.99	4	19
Fig	7.37	3.44	3	16
Gala apple #	6.58	4.41	3	19
Galia melon #	10.59	4.14	4	19
Gooseberry # *	7.86	3.62	5	19
Granny smith #	5.96	3.84	3	15
Grapefruit #	6.58	2.96	3	15
Grape #	4.51	1.32	3	8
Green melon #	6.02	4.24	1	16
Guava # *	12.32	3.86	5	19
Hazelnut	7.17	2.36	4	13

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	max
Honeydew melon #	8.96	4.44	4	19
Horse chestnut *	7.17	2.91	4	13
Jaffa # *	12.02	4.68	5	19
Kiwi #	5.96	2.25	2	12
Kumquat # *	11.76	3.89	6	19
Lemon #	4.44	1.92	1	10
Lime # *	6.05	2.43	3	12
Loganberry	11.32	4.10	5	20
Lychee #	10.63	4.06	5	19
Mandarin # ~	6.84	4.00	2	16
Mango #	7.24	3.25	4	17
Melon #	4.75	1.78	1	8
Nectarine #	5.82	2.49	3	13
Nut	4.64	2.92	1	13
Olive # ~ *	7.37	2.98	3	14
Orange # *	3.66	1.26	1	8
Papaya #	10.60	3.72	6	20
Passion fruit #	8.61	3.20	3	17
Peach #	5.29	1.93	2	10
Pear #	4.23	1.87	1	10
Pepper # *	6.41	2.88	3	14
Pineapple #	5.52	1.79	3	9
Plum	5.55	2.36	2	13
Pomegranate	10.08	4.40	6	19
Prune	7.53	3.01	3	14
Raisin # ~ *	4.92	1.89	3	10
Raspberry #	4.77	2.05	1	9
Red currant	8.11	2.71	4	16
Redberry	7.62	4.31	4	19
Red grape #	5.96	2.17	3	12
Rhubarb	6.26	2.52	4	14
Satsuma #	6.13	2.46	4	15
Squash #	8.44	3.99	4	18
Star fruit #	9.72	4.04	5	18
Strawberry #	4.57	1.23	3	8
Sultana *	5.58	2.72	2	14
Tangerine #	5.98	2.38	3	12
Tomato #	4.02	1.93	1	11
Walnut	7.34	2.12	4	14

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	max
Water melon #	5.80	3.14	3	14
White grape# ~	5.97	3.83	3	18

Table A.2a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to the 21-30 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark #	7.54	2.95	5	16
Adder # ~	6.89	2.46	4	14
Albatross # ~	7.69	2.74	4	16
Alligator # *	5.61	1.90	3	10
Amoeba	10.83	2.38	7	18
Angel fish #	9.95	5.04	5	26
Ant # *	4.07	2.13	2	13
Anteater # ~	7.13	2.60	3	15
Antelope # ~	8.07	2.39	5	13
Ape #	5.81	2.47	3	12
Armadillo #	8.18	2.42	5	14
Ass #	7.72	2.57	4	15
Baboon #	6.77	2.53	3	14
Badger #	5.48	2.97	3	20
Bald eagle #	7.27	1.81	4	11
Bat #	5.10	1.80	3	10
Bear *	3.90	1.85	2	8
Beaver # *	5.79	2.29	4	12
Bee # *	3.67	1.22	2	6
Beetle	4.27	2.17	2	12
Bird	3.26	1.22	2	6
Bison ~	8.88	3.69	4	19
Blackbird	4.99	2.16	3	11
Blue bird #	5.88	2.52	3	13
Blue tit # *	6.59	2.40	3	14
Boa constrictor #	7.49	3.16	3	18
Boar	7.79	2.64	4	15
Brontosaurus # ~	6.67	2.32	3	14
Brown bear #	5.42	1.89	2	10
Buck	9.94	3.34	6	18
Budgerigar #	6.49	2.73	3	15
Buffalo #	7.73	2.36	5	15
Bull	5.29	2.45	3	12
Bullfinch	8.93	2.84	4	15
Bullock	7.08	3.29	3	18

Animal	Mean	SD	Range min	Range max
Butterfly	4.04	2.69	2	18
Buzzard	8.38	2.92	4	17
Calf	5.11	1.76	3	10
Camel	5.56	1.53	4	9
Canary	6.04	3.03	2	16
Caribou	11.43	4.97	7	26
Carp # ~	7.41	3.03	3	17
Cat	3.18	1.24	2	6
Caterpillar	4.22	1.41	2	8
Cattle	4.98	1.74	2	8
Chaffinch	8.66	2.98	5	17
Chameleon #	7.53	2.45	4	17
Cheetah # ~	5.96	2.58	3	17
Chicken # *	3.47	1.33	1	7
Chimpanzee	5.07	2.20	2	11
Chinchilla #	8.95	2.06	4	13
Chipmunk # ~ *	6.24	2.28	3	13
Clown fish #	11.62	5.24	5	23
Cobra # ~	7.26	1.90	5	12
Cockatiel #	6.58	3.12	2	13
Cocker spaniel	6.71	2.71	3	12
Cockerel	4.80	3.51	1	20
Cockroach	7.02	2.69	4	16
Cod	6.07	1.80	3	10
Condor # ~	9.43	2.98	5	18
Conger eel	10.23	4.38	6	21
Cougar # ~	8.48	3.77	4	18
Cow *	3.61	1.37	2	7
Coyote # ~ *	8.17	2.73	4	15
Cray fish # ~	10.76	5.08	4	27
Crocodile #	4.66	2.11	2	11
Crow	5.40	1.41	3	8
Cuckoo	5.86	2.16	3	11
Deer # *	5.29	2.34	3	15
Dingo	9.48	3.47	5	18
Dog	2.72	1.47	1	9
Dolphin #	4.83	1.79	2	9
Donkey	4.30	2.20	2	13
Dormouse	6.70	2.01	4	12

Animal	Mean	SD	Range min	Range max
Dove #	5.49	2.23	2	12
Dragon #	4.74	1.58	2	9
Dragonfly # *	6.44	2.19	4	13
Duck # *	3.52	1.44	2	8
Duckbill platypus #	8.94	2.74	3	14
Eagle	5.36	1.70	2	10
Earthworm	4.65	2.41	2	13
Earwig *	5.54	2.13	3	11
Eel	6.54	2.61	3	12
Elk	9.61	3.43	4	18
Emu #	6.95	3.60	4	20
Ewe	7.09	4.01	3	18
Ferret	6.85	2.5	3	13
Field mouse	6.27	1.96	4	12
Finch	8.14	3.14	4	17
Fish	3.35	1.99	2	13
Flamingo #	6.23	2.19	3	12
Flea	5.83	2.06	3	14
Fly *	3.83	1.22	2	6
Fowl # ~	7.98	3.18	5	16
Fox # *	4.42	1.28	3	9
Frog # *	3.82	2.15	2	14
Gazelle	8.73	3.68	4	22
Gecko #	8.93	4.36	5	20
Gerbil #	5.80	2.06	4	11
Giant panda #	6.31	2.78	3	15
Gibbon #	9.19	3.31	6	18
Giraffe	5.02	2.70	2	18
Goat	4.36	1.68	2	10
Goldfish	4.15	1.54	2	11
Goose #	4.77	1.81	2	10
Gorilla #	5.34	2.97	3	20
Greyhound	6.07	2.81	2	13
Grizzly bear # *	5.36	1.67	3	9
Groundhog # *	10.02	2.50	6	15
Guinea fowl	9.71	4.50	4	21
Guinea pig #	4.91	1.53	3	10
Gull	6.47	2.68	3	13
Haddock	6.94	2.56	4	14

Animal	Mean	SD	Range min	Range max
Hamster #	4.83	1.53	3	10
Hare	5.78	2.27	3	13
Hawk # ~	7.34	2.63	4	16
Hedgehog	4.66	1.76	3	12
Hen	3.97	1.30	2	7
Heron #	6.86	2.81	3	18
Herring	8.44	3.16	5	20
Hippopotamus #	4.73	1.96	2	11
Hornet	6.95	3.41	1	18
Horse	3.58	1.33	2	7
Horsefly	7.99	3.96	4	20
Hyena #	7.13	2.69	4	16
Iguana # *	8.23	2.76	5	16
Insect #	4.50	2.12	2	12
Invertebrate	9.44	2.57	5	16
Jack rabbit	7.04	3.43	3	16
Jackal # ~	9.14	3.01	5	18
Jackass	9.78	3.38	7	20
Jackdaw	7.73	2.91	4	15
Jaguar #	7.26	1.79	5	11
Kangaroo #	4.62	2.25	1	12
Kid	6.31	2.64	3	15
Kitten	3.65	1.89	2	12
Kiwi	9.54	4.44	5	26
Koala #	5.89	2.25	2	12
Koi carp #	8.99	4.86	5	23
Komodo dragon # ~	9.88	4.38	3	21
Lady bird #	3.90	1.37	2	7
Lamb	4.04	1.42	2	8
Lemur #	8.33	2.62	4	14
Leopard #	6.15	2.12	4	12
Lion # *	4.04	1.60	2	8
Lizard #	5.57	1.83	2	10
Llama	8.16	3.69	4	19
Lobster # ~	6.90	2.43	4	12
Long tailed tit	6.30	3.93	1	19
Lynx	9.74	3.35	6	19
Mackerel *	7.54	3.07	3	17
Mammal #	6.33	3.17	2	18

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Meerkat #	9.26	4.94	4	26
Midge	6.49	2.94	2	14
Mink	9.46	3.51	4	19
Manx	9.44	2.83	5	16
Mole	5.37	2.13	2	12
Mongoose ~	9.48	3.37	4	17
Monkey	3.86	2.31	2	13
Moose # *	7.00	2.69	3	16
Moth	5.22	2.40	3	16
Mouse	3.72	1.61	2	9
Mule	7.14	2.59	4	15
Newt	7.04	1.95	4	12
Nightingale	8.01	3.81	5	26
Octopus #	5.15	2.09	3	11
Orang-utan #	6.36	2.59	3	16
Ostrich #	6.35	1.88	3	11
Otter #	6.36	2.38	3	16
Owl	4.76	2.83	3	19
Ox	6.73	2.33	3	12
Panda #	5.12	2.11	2	10
Panther # ~	6.41	1.89	3	12
Parakeet	8.71	2.50	5	15
Parrot #	5.10	1.58	2	9
Partridge #	6.34	2.32	3	10
Peacock #	5.00	2.05	2	10
Pelican #	6.74	2.09	4	14
Penguin #	5.01	1.87	2	11
Perch	9.07	2.84	4	14
Pheasant	7.20	2.67	4	15
Pig # *	3.57	1.36	2	8
Pigeon	4.77	1.78	2	10
Piglet ~	4.35	2.74	2	18
Pike # ~	7.53	4.55	3	26
Piranha fish # ~	7.71	2.15	4	13
Plaice #	8.55	3.00	5	17
Platypus #	9.44	2.56	5	14
Polar bear	5.43	2.08	3	13
Pony	4.38	2.53	2	17
Porcupine	7.86	2.39	5	14

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Porpoise	9.23	2.99	5	20
Poultry #	6.74	2.66	2	12
Prairie dog #	9.59	3.67	6	22
Puffin #	6.50	1.73	4	10
Puma #	7.43	2.80	4	15
Rabbit # *	3.64	1.26	2	6
Racoon #	6.97	1.62	5	11
Ram	6.71	2.28	4	13
Rat	4.12	1.46	1	8
Raven ~	7.56	2.54	3	16
Reindeer #	3.68	2.72	2	18
Rhesus monkey	12.64	5.47	6	26
Rhinoceros #	5.78	1.93	3	10
Roach	8.55	2.44	5	15
Robin	4.82	2.08	2	12
Rodent	6.44	2.07	3	12
Rook	8.41	3.13	5	18
Rooster # *	5.84	1.92	4	10
Salamander #	9.32	4.77	5	27
Salmon	7.40	2.31	3	13
Sardine	6.87	2.90	3	17
Sea lion #	6.32	2.72	3	16
Seagull	4.56	2.02	2	11
Seahorse #	5.91	2.51	4	12
Seal #	5.86	1.86	3	11
Shark #	4.82	1.86	2	10
Sheep # *	3.46	1.36	2	8
Shrew	7.72	2.70	4	14
Shrimp	7.26	2.60	3	14
Siamese cat	6.40	2.28	3	12
Siberian tiger #	8.59	3.01	5	16
Skate	8.91	3.65	4	19
Skunk #	6.75	2.45	4	16
Skylark # *	9.32	2.81	4	14
Sloth #	8.59	2.32	5	14
Slug #	3.99	1.41	2	9
Snail	3.82	1.48	2	8
Snake # *	4.20	2.15	2	14
Sole	8.84	2.93	5	17

Animal	Mean	SD	Range min	Range max
Sow #	8.09	3.04	4	16
Sparrow #	5.15	2.07	2	9
Spider	3.52	1.84	2	12
Springbok	9.68	4.06	4	18
Squid #	7.31	2.82	3	15
Squirrel # *	4.45	1.52	2	8
Stag	7.55	2.53	4	15
Star fish #	5.78	2.03	4	13
Starling #	7.86	3.13	3	20
Stick insect #	6.56	2.08	4	13
Stickleback #	7.36	3.58	4	19
Stoat	8.28	2.19	5	13
Sturgeon	11.29	4.66	8	23
Swan	4.31	2.32	2	12
Swift	7.90	2.82	4	14
Sword fish	7.81	3.01	4	16
Tarantula #	6.39	1.84	3	11
Thrush # *	8.77	2.80	4	16
Tiger # *	4.52	2.32	2	11
Toad # *	4.83	1.27	3	8
Tortoise	4.82	2.34	2	14
Trout ~	7.61	2.27	5	13
Tuna #	5.63	2.96	2	14
Turkey # *	4.55	1.50	2	9
Turtle # *	4.98	2.29	3	16
Tyrannosaurus # ~ *	5.84	2.19	3	11
Vole	8.36	3.46	5	20
Vulture # ~	6.49	2.94	3	17
Wallaby #	7.90	2.31	4	13
Walrus	6.94	2.03	4	12
Warthog #	8.28	2.35	4	13
Wasp	4.50	1.99	2	14
Water buffalo #	9.27	3.58	6	23
Water rat	7.89	2.57	3	13
Weasel # *	6.42	2.23	3	12
Whale #	4.57	1.73	1	10
White tiger #	7.69	3.24	4	19
Wild boar #	8.58	3.20	4	21
Wild cat	7.84	2.63	5	14

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Wild dog #	6.83	3.12	2	14
Wildebeest # ~	9.01	2.61	5	15
Wolf	5.19	1.86	2	10
Wombat #	8.14	2.46	3	14
Woodlouse #	5.21	2.70	3	14
Woodpecker	6.35	1.83	4	11
Worm	3.41	1.42	2	7
Wren	8.18	3.32	4	16
Yak #	8.32	2.79	4	15
Yellow tit	8.71	2.70	4	15
Yorkshire terrier *	6.39	2.90	3	14
Zebra # *	5.01	1.56	3	9

Table A.2b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to the 21-30 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	5.31	1.61	2	9
Almond	6.37	2.18	2	10
Apple # *	3.22	1.17	2	6
Apricot #	5.84	1.85	3	12
Aubergine #	9.25	3.72	5	24
Avocado #	8.40	3.02	4	15
Banana # *	3.26	1.17	2	6
Berries	5.03	1.82	3	10
Blackberry	5.33	1.75	3	10
Blackcurrant # *	4.68	1.65	2	9
Blueberry # ~	6.58	2.42	4	13
Bramble ~ *	5.10	2.20	2	12
Cantaloupe melon #	8.64	3.91	4	19
Cherry #	4.82	1.78	2	9
Chestnut	6.48	2.11	4	12
Clementine #	6.26	2.16	2	12
Coconut	5.80	1.85	3	12
Cox apple *	6.31	3.12	2	18
Crab apple	7.20	4.17	3	19
Cranberry #	7.88	3.08	4	20
Cucumber #	4.75	1.91	3	12
Currant	4.82	1.97	2	9
Damson #	11.12	5.52	7	26
Date	7.24	2.34	3	15
Elderberry *	10.09	4.39	4	25
Fig	6.62	3.35	3	18
Gala apple #	6.01	3.14	2	16
Galia melon #	8.09	3.88	4	19
Gooseberry # *	6.38	3.03	3	14
Granny smith #	5.89	1.57	4	9
Grapefruit #	5.79	2.06	2	10
Grape #	4.11	1.41	2	7
Green melon #	6.69	2.07	4	12
Guava # *	11.34	5.45	4	27

Fruit	Mean	SD	Range min	Range max
Hazelnut	6.00	2.50	2	13
Honeydew melon #	7.88	3.75	4	20
Horse chestnut *	7.34	2.11	5	12
Jaffa # *	7.77	3.60	4	21
Kiwi #	6.43	2.46	4	14
Kumquat # *	10.60	5.07	6	26
Lemon #	4.30	1.69	2	9
Lime # *	5.79	2.37	3	11
Loganberry	12.43	5.88	6	28
Lychee #	11.74	5.64	7	29
Mandarin # ~	6.45	2.46	2	12
Mango #	7.13	3.46	4	18
Melon #	4.90	1.58	2	9
Nectarine #	5.91	2.47	2	15
Nut	4.20	1.73	2	9
Olive # ~ *	8.19	2.85	4	15
Orange # *	3.46	1.08	2	6
Papaya #	9.83	4.66	4	22
Passion fruit #	8.71	2.84	5	16
Peach #	5.17	1.64	2	10
Pear #	3.90	1.36	2	7
Pepper # *	7.07	1.82	4	12
Pineapple #	5.28	1.56	2	9
Plum	5.24	2.29	2	13
Pomegranate	8.16	4.07	4	21
Prune	6.62	2.31	4	12
Raisin # ~ *	4.49	1.78	2	9
Raspberry #	4.93	1.45	3	9
Red currant	6.37	3.25	4	20
Redberry	6.69	2.61	4	17
Red grape #	4.93	2.78	2	13
Rhubarb	5.51	2.22	3	12
Satsuma #	5.06	1.92	2	10
Squash #	6.73	5.21	2	21
Star fruit #	11.11	5.41	6	26
Strawberry #	4.00	1.20	2	7
Sultana *	5.27	1.87	3	11
Tangerine #	5.25	1.97	2	10
Tomato #	3.92	1.09	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range	
			min	max
Walnut	5.65	1.93	2	10
Water melon #	6.12	3.11	3	18
White grape# ~	4.75	3.42	2	20

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table A.3a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to the 31-40 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark #	7.80	3.64	4	20
Adder # ~	8.29	2.56	6	14
Albatross # ~	9.62	3.69	5	21
Alligator # *	5.64	1.96	3	10
Amoeba	11.36	4.32	6	23
Angel fish #	11.40	6.80	6	28
Ant # *	3.83	1.45	2	8
Anteater # ~	7.28	1.96	5	12
Antelope # ~	7.17	2.35	5	12
Ape #	6.30	1.60	4	9
Armadillo #	8.83	3.65	5	20
Ass #	7.38	2.87	3	16
Baboon #	6.74	2.41	4	12
Badger #	5.25	2.45	2	12
Bald eagle #	7.87	3.20	4	18
Bat #	5.18	1.87	3	9
Bear *	3.80	1.63	2	8
Beaver # *	7.33	1.93	5	11
Bee # *	3.46	1.18	2	6
Beetle	4.41	2.09	2	9
Bird	2.05	1.00	1	4
Bison ~	7.95	2.97	5	15
Blackbird	4.73	2.03	2	9
Blue bird #	6.43	3.26	3	16
Blue tit # *	4.50	2.66	1	11
Boa constrictor #	7.55	2.57	5	13
Boar	6.44	3.53	1	18
Brontosaurus # ~	7.26	2.30	5	12
Brown bear #	5.11	2.61	3	12
Buck	9.13	3.28	5	18
Budgerigar #	5.76	3.00	3	14
Buffalo #	6.81	2.14	5	12
Bull	5.03	1.99	2	9
Bullfinch	8.75	2.99	4	16
Bullock	9.20	4.79	6	21

Animal	Mean	SD	Range min	Range max
Butterfly	2.82	1.66	1	7
Buzzard	7.18	3.24	2	16
Calf	4.15	1.78	2	8
Camel	4.32	2.16	1	10
Canary	5.74	2.54	2	11
Caribou	10.53	3.52	6	20
Carp # ~	10.81	5.59	5	30
Cat	2.03	1.12	1	5
Caterpillar	4.06	1.31	2	7
Cattle	5.38	2.84	2	12
Chaffinch	7.20	3.36	3	16
Chameleon #	8.29	3.18	6	20
Cheetah # ~	6.58	2.12	4	12
Chicken # *	2.97	1.58	2	8
Chimpanzee	4.94	1.71	2	9
Chinchilla #	11.73	5.89	7	30
Chipmunk # ~ *	7.66	5.45	4	22
Clown fish #	14.99	6.89	9	32
Cobra # ~	7.04	2.11	4	11
Cockatiel #	6.98	2.68	4	12
Cocker spaniel	7.27	2.42	3	12
Cockerel	5.26	3.45	3	16
Cockroach	6.48	3.15	2	15
Cod	6.15	3.26	3	14
Condor # ~	9.28	2.65	6	14
Conger eel	10.13	4.06	5	21
Cougar # ~	9.47	4.25	5	21
Cow *	2.67	1.28	1	6
Coyote # ~ *	7.15	2.55	5	13
Cray fish # ~	11.56	4.59	7	25
Crocodile #	4.05	1.35	2	7
Crow	5.27	1.59	3	8
Cuckoo	5.69	2.25	4	11
Deer # *	5.01	1.74	3	9
Dingo	9.39	4.25	4	21
Dog	2.21	0.90	1	4
Dolphin #	4.40	2.67	2	13
Donkey	3.40	1.52	2	8
Dormouse	6.39	2.32	3	12

Animal	Mean	SD	Range min	Range max
Dove #	5.42	1.90	3	10
Dragon #	4.67	1.87	3	10
Dragonfly # *	5.40	2.22	2	11
Duck # *	2.77	1.50	1	7
Duckbill platypus #	9.50	2.35	7	15
Eagle	5.56	1.84	3	10
Earthworm	3.64	2.27	1	11
Earwig *	5.41	2.01	2	11
Eel	7.04	2.33	3	12
Elk	10.32	3.39	7	20
Emu #	5.97	4.27	3	20
Ewe	7.09	4.11	4	18
Ferret	6.82	3.05	4	14
Field mouse	5.39	1.97	3	10
Finch	7.68	3.06	4	16
Fish	2.86	0.85	2	4
Flamingo #	5.93	2.29	4	11
Flea	5.97	1.62	4	10
Fly *	3.05	1.77	1	8
Fowl # ~	5.82	4.87	2	19
Fox # *	4.28	1.76	2	8
Frog # *	3.53	1.33	2	6
Gazelle	8.75	2.64	6	16
Gecko #	11.76	6.51	6	28
Gerbil #	6.19	2.48	4	14
Giant panda #	6.44	5.95	4	32
Gibbon #	8.44	3.22	5	16
Giraffe	4.08	5.35	2	26
Goat	4.06	1.95	3	10
Goldfish	3.61	2.06	2	10
Goose #	5.32	2.08	3	10
Gorilla #	5.32	1.95	3	10
Greyhound	7.14	3.38	3	15
Grizzly bear # *	5.58	1.86	3	10
Groundhog # *	10.17	5.31	3	27
Guinea fowl	11.28	4.87	6	25
Guinea pig #	5.43	2.12	2	10
Gull	5.70	2.17	3	10
Haddock	6.40	3.58	3	16

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Hamster #	4.92	1.93	3	11
Hare	5.66	1.73	4	9
Hawk # ~	6.90	2.44	3	12
Hedgehog	4.28	2.17	2	10
Hen	3.60	1.70	2	8
Heron #	8.10	3.12	4	14
Herring	8.41	2.18	6	13
Hippopotamus #	4.87	1.64	3	8
Hornet	8.29	3.76	5	20
Horse	2.85	1.58	1	8
Horsefly	9.61	3.78	7	19
Hyena #	7.74	2.43	5	12
Iguana # *	9.24	3.31	4	16
Insect #	4.09	1.94	2	8
Invertebrate	9.20	2.74	6	16
Jack rabbit	7.68	2.20	5	12
Jackal # ~	10.26	3.15	0	18
Jackass	10.50	5.80	6	25
Jackdaw	9.09	7.11	5	37
Jaguar #	7.61	2.43	4	12
Kangaroo #	3.91	2.11	1	8
Kid	5.79	2.66	3	12
Kitten	2.63	1.35	1	6
Kiwi	10.25	5.38	5	22
Koala #	6.10	2.40	4	13
Koi carp #	9.84	4.11	4	19
Komodo dragon # ~	12.23	7.65	5	36
Lady bird #	3.61	1.68	2	8
Lamb	3.61	1.68	2	8
Lemur #	11.12	7.77	5	37
Leopard #	5.78	2.41	3	13
Lion # *	3.83	1.12	2	6
Lizard #	5.84	2.52	3	12
Llama	8.51	3.22	5	18
Lobster # ~	6.94	2.60	4	13
Long tailed tit	11.21	9.02	5	34
Lynx	9.60	5.25	6	30
Mackerel *	8.64	2.91	5	16
Mammal #	6.83	1.57	5	10

Animal	Mean	SD	Range min	max
Meerkat #	12.12	6.00	7	30
Midge	8.26	4.17	5	18
Mink	9.27	3.06	5	15
Manx	9.93	3.72	6	20
Mole	5.82	2.17	3	12
Mongoose ~	8.82	3.11	4	16
Monkey	2.93	1.69	1	8
Moose # *	6.99	2.66	4	13
Moth	5.00	1.71	3	8
Mouse	3.18	1.49	1	7
Mule	6.97	1.90	5	10
Newt	6.56	2.34	4	12
Nightingale	8.32	3.14	5	16
Octopus #	5.83	1.66	3	10
Orang-utan #	6.53	2.15	3	11
Ostrich #	4.95	2.70	1	14
Otter #	6.70	2.30	4	12
Owl	4.41	1.49	2	8
Ox	6.79	3.20	3	14
Panda #	5.03	2.96	3	14
Panther # ~	6.63	1.76	4	11
Parakeet	7.62	3.09	2	18
Parrot #	5.16	1.77	3	10
Partridge #	6.69	3.17	4	14
Peacock #	4.89	2.31	3	12
Pelican #	6.50	2.37	2	12
Penguin #	4.51	1.30	3	8
Perch	8.94	3.27	5	16
Pheasant	6.96	2.60	4	14
Pig # *	2.83	1.58	1	8
Pigeon	4.36	1.25	3	7
Piglet ~	3.59	1.89	2	10
Pike # ~	9.14	2.76	6	16
Piranha fish # ~	7.14	2.48	5	13
Plaice #	9.16	5.01	4	24
Platypus #	9.01	3.21	6	16
Polar bear	5.72	2.11	3	11
Pony	3.84	1.88	2	8
Porcupine	7.37	1.95	4	12

Animal	Mean	SD	Range min	max
Porpoise	8.29	4.42	4	23
Poultry #	7.03	3.06	2	15
Prairie dog #	10.19	3.33	6	18
Puffin #	6.72	3.41	4	16
Puma #	8.25	1.92	6	12
Rabbit # *	3.08	1.24	2	5
Racoon #	7.50	2.61	3	12
Ram	6.87	1.65	5	11
Rat	4.37	1.50	2	8
Raven ~	7.57	2.73	4	14
Reindeer #	3.40	2.11	2	10
Rhesus monkey	11.82	4.92	6	24
Rhinoceros #	5.67	2.00	3	10
Roach	7.61	4.23	3	20
Robin	4.19	2.05	2	9
Rodent	8.36	1.90	5	12
Rook	7.91	2.52	4	15
Rooster # *	5.46	2.64	3	12
Salamander #	9.50	3.65	5	16
Salmon	7.60	3.41	5	15
Sardine	6.60	2.57	2	12
Sea lion #	6.06	2.37	3	12
Seagull	4.51	2.40	3	12
Seahorse #	6.72	2.31	5	14
Seal #	5.45	2.21	2	11
Shark #	4.75	1.77	3	10
Sheep # *	3.06	1.39	2	7
Shrew	9.08	2.77	6	15
Shrimp	7.23	2.37	3	12
Siamese cat	6.47	1.84	5	10
Siberian tiger #	9.98	8.00	6	40
Skate	10.41	4.17	7	22
Skunk #	6.69	1.80	5	11
Skylark # *	8.42	3.33	5	16
Sloth #	9.64	4.47	6	22
Slug #	4.24	1.39	2	8
Snail	3.43	1.24	2	6
Snake # *	4.31	1.21	3	7
Sole	9.89	3.14	7	16

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Sow #	7.20	3.16	3	15
Sparrow #	5.02	2.43	3	12
Spider	2.72	1.15	1	5
Springbok	10.08	3.35	8	18
Squid #	6.87	2.83	4	12
Squirrel # *	3.61	1.89	1	8
Stag	7.57	3.31	4	16
Star fish #	4.69	2.36	2	11
Starling #	6.76	3.61	3	16
Stick insect #	6.51	3.20	4	18
Stickleback #	7.52	4.10	4	20
Stoat	9.38	2.69	6	15
Sturgeon	13.18	5.08	8	31
Swan	4.01	1.54	2	8
Swift	7.99	3.05	5	16
Sword fish	8.44	3.18	5	18
Tarantula #	6.59	2.59	4	14
Thrush # *	6.39	3.61	2	16
Tiger # *	4.01	1.64	2	8
Toad # *	4.81	1.51	3	8
Tortoise	3.79	1.73	1	8
Trout ~	7.73	2.71	4	15
Tuna #	6.76	2.72	3	14
Turkey # *	4.33	1.83	2	10
Turtle # *	5.09	1.84	2	9
Tyrannosaurus # ~ *	6.42	2.07	4	10
Vole	8.74	2.83	5	16
Vulture # ~	6.59	2.02	4	10
Wallaby #	7.43	5.49	4	27
Walrus	7.62	2.45	5	13
Warthog #	8.64	3.51	5	15
Wasp	4.08	1.35	3	8
Water buffalo #	9.43	2.88	5	16
Water rat	9.29	2.77	5	16
Weasel # *	6.67	3.14	3	13
Whale #	4.74	1.62	3	8
White tiger #	9.02	6.02	6	30
Wild boar #	9.32	2.56	7	14
Wild cat	9.19	3.08	6	16

Animal	Mean	SD	Range min	Range max
Wild dog #	7.94	2.79	5	14
Wildebeest # ~	8.44	3.35	5	18
Wolf	4.27	2.14	2	10
Wombat #	9.64	5.73	5	30
Woodlouse #	4.17	2.40	2	10
Woodpecker	5.80	1.86	3	10
Worm	3.00	1.64	1	7
Wren	7.90	3.47	4	16
Yak #	9.66	2.98	5	17
Yellow tit	9.31	8.17	5	40
Yorkshire terrier *	6.38	5.15	3	25
Zebra # *	5.13	1.58	3	9

Table A.3b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to the 31-40 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	5.21	1.96	3	10
Almond	6.29	2.65	4	14
Apple # *	2.37	1.46	1	6
Apricot #	6.69	3.06	4	15
Aubergine #	10.58	5.33	6	25
Avocado #	8.27	4.80	4	20
Banana # *	2.48	1.81	1	8
Berries	4.47	2.10	2	9
Blackberry	5.54	2.45	4	12
Blackcurrant # *	5.30	2.45	0	10
Blueberry # ~	8.34	4.13	4	20
Bramble ~ *	5.15	3.62	3	15
Cantaloupe melon #	9.46	9.86	4	34
Cherry #	5.03	1.88	3	10
Chestnut	6.40	3.32	3	16
Clementine #	7.31	4.23	4	22
Coconut	4.46	2.27	2	10
Cox apple *	7.00	2.97	4	15
Crab apple	6.78	2.91	5	15
Cranberry #	10.01	4.99	5	22
Cucumber #	4.18	2.03	2	10
Currant	4.77	2.59	2	12
Damson #	7.97	7.41	3	28
Date	7.51	2.16	4	12
Elderberry *	7.72	3.77	4	18
Fig	7.16	6.39	4	30
Gala apple #	9.07	7.78	4	33
Galia melon #	10.62	10.78	5	40
Gooseberry # *	5.46	3.22	4	17
Granny smith #	6.96	3.45	4	15
Grapefruit #	5.78	2.68	3	15
Grape #	3.62	1.96	2	8
Green melon #	7.30	10.76	3	40
Guava # *	10.25	8.27	4	35

Fruit	Mean	SD	Range min	Range max
Hazelnut	5.96	2.71	3	12
Honeydew melon #	8.45	9.90	3	40
Horse chestnut *	6.51	3.91	3	19
Jaffa # *	5.93	2.33	3	12
Kiwi #	9.08	5.63	4	25
Kumquat # *	10.15	10.38	2	40
Lemon #	4.47	1.74	2	8
Lime # *	6.71	3.53	3	18
Loganberry	12.02	7.24	6	30
Lychee #	12.31	8.95	8	40
Mandarin # ~	6.71	2.16	4	12
Mango #	7.68	5.15	2	25
Melon #	5.29	3.66	3	16
Nectarine #	6.94	2.20	4	12
Nut	3.54	2.21	1	10
Olive # ~ *	9.99	4.33	5	21
Orange # *	2.44	1.45	1	6
Papaya #	8.07	7.31	2	30
Passion fruit #	11.09	5.85	6	25
Peach #	4.72	2.72	2	12
Pear #	3.40	1.83	1	8
Pepper # *	6.31	3.35	3	14
Pineapple #	5.22	2.13	3	10
Plum	4.80	2.04	3	10
Pomegranate	7.28	5.40	4	25
Prune	6.23	2.25	3	11
Raisin # ~ *	4.37	2.02	2	10
Raspberry #	4.72	2.16	3	10
Red currant	6.98	3.17	4	15
Redberry	7.10	4.01	4	18
Red grape #	5.20	5.47	2	28
Rhubarb	4.41	3.80	1	18
Satsuma #	4.57	3.32	3	15
Squash #	9.28	11.57	3	40
Star fruit #	12.66	9.14	4	40
Strawberry #	3.47	2.14	1	8
Sultana *	5.28	2.38	2	12
Tangerine #	4.61	2.39	2	10
Tomato #	2.76	1.75	1	8

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range	
			min	max
Walnut	6.43	1.49	5	10
Water melon #	6.14	3.60	3	18
White grape# ~	5.55	5.73	3	28

Table A.4a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to the 41-50 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark #	9.21	3.18	5	16
Adder # ~	7.56	3.50	5	16
Albatross # ~	8.33	2.21	5	14
Alligator # *	5.50	2.01	3	10
Amoeba	11.09	1.99	8	15
Angel fish #	8.87	9.47	4	43
Ant # *	3.84	0.88	3	6
Anteater # ~	7.04	2.10	5	13
Antelope # ~	8.26	2.56	5	13
Ape #	5.92	1.78	4	10
Armadillo #	8.35	3.56	5	20
Ass #	7.44	2.79	5	13
Baboon #	6.72	2.59	4	12
Badger #	6.25	2.91	4	14
Bald eagle #	9.5	2.7	5	14
Bat #	5.11	1.89	3	9
Bear *	3.63	1.54	2	8
Beaver # *	7.41	2.11	5	13
Bee # *	3.65	1.66	2	8
Beetle	5.12	1.78	3	9
Bird	2.83	1.03	2	5
Bison ~	9.35	5.19	6	26
Blackbird	4.68	3.13	2	15
Blue bird #	7.23	2.39	5	12
Blue tit # *	6.84	3.51	4	16
Boa constrictor #	8.32	1.60	7	12
Boar	8.36	2.96	5	16
Brontosaurus # ~	8.23	2.99	6	16
Brown bear #	6.79	3.28	4	14
Buck	9.13	4.35	5	20
Budgerigar #	5.66	3.85	3	18
Buffalo #	7.34	2.50	4	12
Bull	5.56	1.41	4	8
Bullfinch	8.91	5.29	6	25
Bullock	7.53	3.13	5	14

Animal	**Mean**	**SD**	**Range** min	max
Butterfly	3.75	2.06	2	9
Buzzard	9.01	4.34	6	22
Calf	5.40	2.18	3	12
Camel	5.21	1.82	3	10
Canary	7.33	2.75	4	13
Caribou	12.20	3.80	8	24
Carp # ~	9.59	3.56	6	18
Cat	2.88	0.83	2	5
Caterpillar	4.32	1.96	3	9
Cattle	5.55	3.17	3	14
Chaffinch	9.15	3.31	6	18
Chameleon #	9.54	3.09	7	18
Cheetah # ~	6.20	2.50	3	14
Chicken # *	4.03	1.39	3	8
Chimpanzee	5.79	2.24	3	10
Chinchilla #	11.29	5.66	7	26
Chipmunk # ~ *	7.93	2.02	6	12
Clown fish #	15.30	11.17	8	40
Cobra # ~	7.04	2.63	4	13
Cockatiel #	9.80	2.04	7	13
Cocker spaniel	7.69	2.98	4	15
Cockerel	5.94	3.08	4	14
Cockroach	7.54	2.04	5	12
Cod	6.65	3.73	4	16
Condor # ~	10.41	3.56	7	19
Conger eel	10.53	5.70	7	30
Cougar # ~	9.76	2.12	6	14
Cow *	3.44	0.84	2	5
Coyote # ~ *	9.05	3.21	7	15
Cray fish # ~	11.88	4.55	6	24
Crocodile #	5.17	2.54	3	11
Crow	5.64	2.56	3	12
Cuckoo	5.07	2.58	2	11
Deer # *	5.35	2.06	3	10
Dingo	9.20	3.05	6	15
Dog	2.86	1.03	2	5
Dolphin #	5.35	2.42	4	12
Donkey	3.82	1.79	2	8
Dormouse	6.23	2.59	3	13

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Dove #	6.34	2.08	3	10
Dragon #	4.68	1.72	3	9
Dragonfly # *	6.93	3.12	3	12
Duck # *	3.69	2.50	2	12
Duckbill platypus #	9.07	3.26	6	15
Eagle	5.23	2.11	3	10
Earthworm	3.73	2.74	2	10
Earwig *	5.35	2.58	3	11
Eel	6.79	3.85	5	18
Elk	10.47	5.53	7	28
Emu #	9.01	2.79	5	16
Ewe	7.15	1.43	5	10
Ferret	8.35	3.60	6	20
Field mouse	6.84	3.11	3	14
Finch	8.65	3.08	6	14
Fish	3.51	1.05	2	6
Flamingo #	8.19	2.02	5	12
Flea	5.85	2.28	4	12
Fly *	3.64	1.64	2	9
Fowl # ~	8.60	3.38	5	16
Fox # *	5.35	1.63	3	8
Frog # *	3.62	1.11	2	5
Gazelle	8.68	2.93	6	14
Gecko #	12.53	4.09	8	20
Gerbil #	6.70	5.11	3	24
Giant panda #	7.65	3.47	5	16
Gibbon #	6.58	4.79	2	18
Giraffe	5.06	1.82	3	9
Goat	4.09	1.60	2	8
Goldfish	4.18	1.66	2	8
Goose #	5.26	2.59	3	12
Gorilla #	5.73	1.49	4	8
Greyhound	7.90	2.62	5	14
Grizzly bear # *	5.99	2.64	2	12
Groundhog # *	12.85	5.88	8	29
Guinea fowl	10.41	4.79	6	26
Guinea pig #	6.51	2.67	4	14
Gull	5.80	2.94	4	12
Haddock	6.79	3.29	3	15

Animal	Mean	SD	Range min	max
Hamster #	4.83	1.42	3	8
Hare	5.79	2.84	4	12
Hawk # ~	7.62	2.84	5	16
Hedgehog	4.86	2.41	3	12
Hen	4.09	1.82	2	8
Heron #	8.51	2.37	6	14
Herring	6.78	4.41	3	18
Hippopotamus #	5.50	1.64	3	10
Hornet	7.06	2.03	5	12
Horse	3.46	1.44	2	7
Horsefly	9.59	9.59	6	44
Hyena #	8.34	3.30	5	16
Iguana # *	10.69	2.55	8	16
Insect #	4.68	1.66	3	9
Invertebrate	10.52	8.65	7	40
Jack rabbit	8.37	4.77	5	22
Jackal # ~	9.03	3.19	6	18
Jackass	11.54	7.86	6	30
Jackdaw	8.62	3.04	5	16
Jaguar #	8.15	3.40	5	16
Kangaroo #	5.29	2.40	3	12
Kid	7.48	3.91	5	16
Kitten	3.48	1.46	2	8
Kiwi	10.33	4.17	6	21
Koala #	6.42	2.68	3	12
Koi carp #	12.21	6.08	6	28
Komodo dragon # ~	14.22	3.89	11	24
Lady bird #	4.02	1.74	2	8
Lamb	3.75	1.65	2	7
Lemur #	11.04	3.29	7	17
Leopard #	5.69	2.54	3	12
Lion # *	3.35	1.57	2	6
Lizard #	6.22	2.12	4	10
Llama	9.71	3.06	6	16
Lobster # ~	8.82	1.83	7	12
Long tailed tit	10.01	4.72	4	20
Lynx	10.52	2.62	8	16
Mackerel *	8.35	3.41	5	17
Mammal #	7.97	2.30	5	12

Animal	Mean	SD	Range min	max
Meerkat #	12.37	10.58	7	40
Midge	7.71	3.88	5	18
Mink	8.53	4.78	5	20
Manx	10.47	5.05	6	25
Mole	4.14	2.14	1	10
Mongoose ~	8.70	2.14	6	14
Monkey	4.05	1.45	2	8
Moose # *	8.41	2.27	5	14
Moth	5.48	1.48	4	9
Mouse	3.50	1.12	2	6
Mule	7.16	2.57	3	12
Newt	7.20	3.92	5	16
Nightingale	7.97	3.79	5	17
Octopus #	6.07	2.10	4	12
Orang-utan #	7.86	2.95	5	14
Ostrich #	6.78	2.71	4	12
Otter #	6.98	3.08	4	14
Owl	4.62	2.18	3	12
Ox	6.68	2.53	3	12
Panda #	4.91	2.17	3	10
Panther # ~	7.26	2.95	3	12
Parakeet	10.14	3.68	7	21
Parrot #	4.72	2.59	3	12
Partridge #	7.42	3.84	4	19
Peacock #	6.24	3.06	4	16
Pelican #	7.71	2.93	5	15
Penguin #	5.32	1.92	3	10
Perch	9.95	3.18	7	16
Pheasant	7.89	3.37	4	15
Pig # *	3.69	1.30	3	7
Pigeon	5.21	1.45	4	9
Piglet ~	4.51	2.05	2	9
Pike # ~	9.49	4.05	5	18
Piranha fish # ~	8.58	2.25	6	12
Plaice #	8.18	5.68	4	26
Platypus #	9.00	2.31	5	14
Polar bear	5.74	2.73	3	12
Pony	4.51	1.71	3	9
Porcupine	7.74	2.71	5	14

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Porpoise	10.16	2.89	7	15
Poultry #	8.46	3.05	6	15
Prairie dog #	11.89	5.56	8	25
Puffin #	7.03	3.11	4	14
Puma #	8.87	2.79	6	15
Rabbit # *	3.46	1.29	2	7
Racoon #	8.59	2.98	5	16
Ram	7.05	2.60	4	12
Rat	4.68	2.05	3	10
Raven ~	7.62	3.56	5	16
Reindeer #	3.50	3.15	2	14
Rhesus monkey	11.31	4.64	7	25
Rhinoceros #	6.16	2.68	3	12
Roach	11.38	4.68	7	24
Robin	4.74	3.15	3	15
Rodent	7.55	2.25	5	12
Rook	5.51	4.27	1	17
Rooster # *	6.60	2.70	4	15
Salamander #	11.04	3.86	5	19
Salmon	7.41	3.30	5	16
Sardine	6.80	3.63	4	18
Sea lion #	7.67	2.50	5	12
Seagull	5.14	1.40	4	8
Seahorse #	6.24	2.44	4	12
Seal #	5.66	2.50	3	11
Shark #	5.82	1.41	4	8
Sheep # *	3.66	1.36	2	8
Shrew	7.92	4.87	3	18
Shrimp	7.99	3.58	5	17
Siamese cat	7.79	2.09	5	12
Siberian tiger #	11.57	3.84	8	20
Skate	9.37	5.61	6	25
Skunk #	6.28	2.20	4	10
Skylark # *	9.43	4.72	7	22
Sloth #	10.92	3.10	7	18
Slug #	5.21	2.28	3	12
Snail	3.86	1.23	3	7
Snake # *	4.43	1.37	3	8
Sole	9.24	3.56	4	16

Animal	Mean	SD	Range min	max
Sow #	7.67	3.83	5	16
Sparrow #	5.12	2.02	3	10
Spider	3.53	1.03	2	5
Springbok	11.23	7.46	5	30
Squid #	7.85	2.97	3	12
Squirrel # *	4.67	1.54	3	8
Stag	7.28	3.38	3	14
Star fish #	5.80	2.31	3	12
Starling #	6.89	3.70	4	16
Stick insect #	8.69	3.02	6	16
Stickleback #	7.87	7.11	5	30
Stoat	9.45	3.57	6	17
Sturgeon	11.55	3.97	7	20
Swan	4.55	1.99	3	10
Swift	7.46	3.74	3	15
Sword fish	8.67	1.80	6	12
Tarantula #	8.48	1.81	7	12
Thrush # *	7.33	3.67	5	16
Tiger # *	4.30	1.16	3	7
Toad # *	5.67	2.24	3	11
Tortoise	4.55	1.98	2	9
Trout ~	7.08	5.93	4	26
Tuna #	7.75	3.99	3	18
Turkey # *	4.69	2.10	2	10
Turtle # *	5.83	2.81	4	12
Tyrannosaurus # ~ *	7.16	2.49	5	12
Vole	8.16	5.05	5	24
Vulture # ~	6.28	2.41	3	10
Wallaby #	7.69	3.15	5	14
Walrus	7.26	3.00	5	14
Warthog #	9.78	3.06	7	16
Wasp	4.17	1.29	3	7
Water buffalo #	9.62	4.16	6	22
Water rat	8.72	3.71	5	16
Weasel # *	8.16	3.05	6	16
Whale #	5.10	1.91	3	10
White tiger #	13.04	5.24	7	28
Wild boar #	9.00	2.89	6	14
Wild cat	9.86	3.61	7	18

Animal	Mean	SD	Range min	max
Wild dog #	9.46	3.88	6	20
Wildebeest # ~	9.86	3.28	6	16
Wolf	4.71	1.70	3	8
Wombat #	8.68	4.14	6	22
Woodlouse #	8.29	2.54	5	13
Woodpecker	6.88	2.65	5	12
Worm	3.44	1.07	2	6
Wren	7.98	4.14	5	18
Yak #	10.53	3.75	6	20
Yellow tit	9.50	3.43	7	17
Yorkshire terrier *	7.70	3.47	5	15
Zebra # *	5.03	2.71	3	12

Table A.4b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to the 41-50 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	5.84	6.12	3	28
Almond	6.46	3.08	4	16
Apple # *	3.61	1.14	2	6
Apricot #	6.97	5.23	4	24
Aubergine #	12.07	2.70	8	16
Avocado #	11.42	4.36	8	23
Banana # *	3.76	1.48	2	7
Berries	5.18	3.52	3	14
Blackberry	5.70	2.77	4	14
Blackcurrant # *	6.42	3.08	4	14
Blueberry # ~	9.27	5.09	5	20
Bramble ~ *	6.54	8.21	4	32
Cantaloupe melon #	12.71	3.28	8	21
Cherry #	5.44	2.18	3	10
Chestnut	6.90	4.94	4	23
Clementine #	8.58	3.39	5	16
Coconut	5.86	4.31	4	21
Cox apple *	7.33	4.88	4	18
Crab apple	7.45	4.22	4	20
Cranberry #	12.79	7.05	7	30
Cucumber #	5.01	2.48	3	10
Currant	5.33	2.30	3	10
Damson #	9.69	5.05	5	20
Date	6.10	2.38	3	12
Elderberry *	8.32	4.28	5	19
Fig	7.47	5.71	5	24
Gala apple #	12.61	8.51	8	38
Galia melon #	13.37	6.13	7	30
Gooseberry # *	6.43	3.57	4	16
Granny smith #	7.44	2.21	5	12
Grapefruit #	6.07	2.33	4	12
Grape #	5.29	1.50	3	9
Green melon #	8.87	8.31	5	35
Guava # *	17.11	8.78	8	40

Fruit	Mean	SD	Range min	Range max
Hazelnut	6.29	3.39	3	14
Honeydew melon #	9.79	5.99	5	26
Horse chestnut *	6.63	4.78	4	19
Jaffa # *	7.21	3.65	4	16
Kiwi #	14.36	4.63	8	23
Kumquat # *	19.09	9.52	11	40
Lemon #	4.80	1.75	3	8
Lime # *	6.68	2.37	5	12
Loganberry	11.35	8.95	6	35
Lychee #	13.29	8.61	7	40
Mandarin # ~	7.16	3.12	4	14
Mango #	11.51	4.22	8	22
Melon #	7.15	2.78	5	14
Nectarine #	10.28	2.27	8	15
Nut	4.34	1.92	3	10
Olive # ~ *	10.86	5.49	5	22
Orange # *	3.96	1.22	2	6
Papaya #	16.55	7.93	9	32
Passion fruit #	12.86	9.65	5	40
Peach #	6.07	1.99	4	10
Pear #	5.00	1.60	3	8
Pepper # *	9.21	3.96	5	18
Pineapple #	4.35	3.12	1	12
Plum	5.66	2.53	4	12
Pomegranate	6.87	4.58	3	22
Prune	6.74	2.27	5	12
Raisin # ~ *	5.34	1.81	4	10
Raspberry #	5.69	2.12	4	11
Red currant	9.72	8.40	5	38
Redberry	6.95	2.70	4	11
Red grape #	6.56	4.91	4	22
Rhubarb	5.36	1.96	3	10
Satsuma #	6.60	2.68	4	13
Squash #	19.53	10.04	9	40
Star fruit #	19.78	7.80	12	35
Strawberry #	4.42	1.69	3	8
Sultana *	5.87	3.03	4	13
Tangerine #	5.64	3.20	3	14
Tomato #	4.56	1.44	3	8

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	**Mean**	**SD**	**Range**	
			min	**max**
Walnut	6.82	2.82	5	13
Water melon #	8.41	4.23	5	20
White grape# ~	7.39	6.70	4	24

Table A.5a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to the 51-60 age –category.

Animal	**Mean**	**SD**	**Range** min	**Range** max
Aardvark #	6.61	5.64	1	21
Adder # ~	7.95	2.28	5	12
Albatross # ~	10.75	2.87	6	15
Alligator # *	7.47	2.24	5	14
Amoeba	12.07	12.77	5	60
Angel fish #	13.64	6.64	7	30
Ant # *	5.47	1.89	3	9
Anteater # ~	9.62	3.83	5	18
Antelope # ~	8.89	3.73	6	19
Ape #	6.72	2.50	4	12
Armadillo #	6.90	3.43	1	15
Ass #	7.53	3.03	4	15
Baboon #	8.70	4.55	5	20
Badger #	6.87	2.86	4	14
Bald eagle #	10.61	4.78	4	21
Bat #	6.80	1.84	4	10
Bear *	4.66	2.17	2	10
Beaver # *	7.69	2.50	5	13
Bee # *	4.70	1.80	3	9
Beetle	5.18	1.80	3	9
Bird	3.48	1.75	2	8
Bison ~	6.75	4.57	1	20
Blackbird	5.75	1.99	3	11
Blue bird #	6.30	4.97	1	20
Blue tit # *	8.28	3.36	4	18
Boa constrictor #	9.28	4.60	6	24
Boar	8.89	4.15	3	20
Brontosaurus # ~	5.86	3.45	1	14
Brown bear #	7.28	2.78	4	15
Buck	9.44	7.17	0	30
Budgerigar #	4.01	2.50	1	10
Buffalo #	7.98	2.52	5	13
Bull	5.67	2.09	3	10
Bullfinch	7.32	6.91	1	30
Bullock	5.63	3.94	1	17

Animal	Mean	SD	Range min	Range max
Butterfly	4.39	1.55	2	8
Buzzard	8.82	4.97	2	21
Calf	5.99	1.95	3	11
Camel	6.45	2.57	4	14
Canary	6.78	2.54	3	12
Caribou	7.47	7.71	1	30
Carp # ~	10.20	8.88	6	40
Cat	3.48	1.50	2	7
Caterpillar	4.96	1.76	3	10
Cattle	6.34	2.39	4	12
Chaffinch	6.83	4.25	1	20
Chameleon #	10.07	4.45	5	22
Cheetah # ~	8.18	3.02	4	14
Chicken # *	4.78	1.74	3	9
Chimpanzee	6.40	1.92	4	12
Chinchilla #	11.13	10.22	4	45
Chipmunk # ~ *	9.76	6.76	5	30
Clown fish #	11.30	15.79	3	60
Cobra # ~	9.11	3.08	6	15
Cockatiel #	9.87	5.01	6	25
Cocker spaniel	7.66	2.22	4	12
Cockerel	5.81	2.48	3	10
Cockroach	10.81	5.27	6	24
Cod	6.23	2.93	4	14
Condor # ~	11.60	8.27	5	40
Conger eel	11.30	5.39	5	25
Cougar # ~	6.64	5.10	1	20
Cow *	4.19	1.74	3	8
Coyote # ~ *	7.01	4.65	1	20
Cray fish # ~	12.15	9.64	5	40
Crocodile #	6.40	1.73	5	10
Crow	6.58	2.17	4	13
Cuckoo	5.75	2.61	2	12
Deer # *	6.29	2.53	3	12
Dingo	9.59	5.01	3	25
Dog	2.88	1.74	1	7
Dolphin #	7.45	2.24	5	12
Donkey	5.03	2.21	3	10
Dormouse	6.35	2.99	3	14

Animal	Mean	SD	Range min	Range max
Dove #	7.14	2.70	3	13
Dragon #	6.12	2.69	3	15
Dragonfly # *	8.22	3.02	5	15
Duck # *	4.35	1.32	2	8
Duckbill platypus #	10.64	5.63	6	23
Eagle	7.31	3.04	5	16
Earthworm	4.86	3.59	2	14
Earwig *	4.48	2.98	1	14
Eel	7.65	3.07	5	16
Elk	7.44	7.23	1	30
Emu #	9.17	3.66	5	18
Ewe	7.43	2.76	5	12
Ferret	8.19	3.13	5	14
Field mouse	7.03	4.64	4	20
Finch	5.99	4.32	1	20
Fish	3.70	1.75	2	8
Flamingo #	8.04	3.17	5	15
Flea	7.56	3.01	4	15
Fly *	4.00	1.90	2	9
Fowl # ~	7.23	3.69	3	16
Fox # *	5.71	1.59	3	9
Frog # *	5.11	1.47	3	9
Gazelle	9.38	4.03	5	20
Gecko #	12.36	6.85	7	30
Gerbil #	9.27	4.05	4	19
Giant panda #	8.18	5.03	4	20
Gibbon #	8.96	4.40	2	20
Giraffe	5.92	3.02	3	14
Goat	5.61	1.91	4	10
Goldfish	4.38	1.75	3	8
Goose #	6.05	2.47	4	12
Gorilla #	6.85	2.43	4	14
Greyhound	7.72	3.72	5	18
Grizzly bear # *	6.66	3.97	3	18
Groundhog # *	14.28	9.90	5	34
Guinea fowl	10.59	6.55	5	30
Guinea pig #	6.34	2.63	4	12
Gull	5.94	2.68	2	12
Haddock	6.00	2.53	3	12

Animal	Mean	SD	Range min	Range max
Hamster #	6.65	2.07	4	10
Hare	6.92	2.72	4	14
Hawk # ~	8.63	3.16	5	15
Hedgehog	4.99	1.99	2	10
Hen	4.69	1.44	2	8
Heron #	7.83	3.36	2	15
Herring	8.13	2.99	4	15
Hippopotamus #	6.53	2.28	4	12
Hornet	9.27	5.71	6	30
Horse	4.51	1.57	3	8
Horsefly	9.04	6.49	5	30
Hyena #	8.14	2.78	4	14
Iguana # *	8.82	5.09	2	22
Insect #	4.73	2.25	2	9
Invertebrate	9.84	3.82	4	16
Jack rabbit	9.18	7.30	2	30
Jackal # ~	11.12	3.90	7	20
Jackass	11.36	5.95	5	25
Jackdaw	6.65	5.70	1	26
Jaguar #	8.37	3.62	5	18
Kangaroo #	6.26	2.28	3	12
Kid	8.31	4.03	5	20
Kitten	4.04	1.86	2	8
Kiwi	10.23	4.35	5	20
Koala #	6.83	3.34	3	14
Koi carp #	14.38	10.68	7	40
Komodo dragon # ~	13.81	9.08	6	35
Lady bird #	4.65	1.46	3	9
Lamb	4.32	1.81	2	8
Lemur #	13.68	7.13	7	30
Leopard #	7.30	2.24	5	14
Lion # *	5.39	1.52	3	9
Lizard #	7.50	2.27	4	13
Llama	9.50	3.30	5	15
Lobster # ~	9.53	4.14	6	20
Long tailed tit	10.46	6.45	5	30
Lynx	6.60	4.43	1	20
Mackerel *	8.58	5.23	4	20
Mammal #	8.44	2.82	5	15

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Meerkat #	14.20	10.99	5	45
Midge	5.07	6.58	1	30
Mink	9.65	3.92	5	19
Manx	6.03	4.85	1	20
Mole	6.72	2.38	5	12
Mongoose ~	9.75	4.49	5	20
Monkey	5.29	1.50	3	9
Moose # *	9.50	2.86	6	15
Moth	5.91	2.06	3	10
Mouse	4.88	1.64	3	9
Mule	7.11	3.17	5	16
Newt	6.26	3.01	2	16
Nightingale	7.81	3.25	5	17
Octopus #	6.16	1.98	3	10
Orang-utan #	8.67	2.97	4	16
Ostrich #	7.96	3.11	4	14
Otter #	8.22	2.65	4	14
Owl	6.02	1.95	4	11
Ox	7.55	2.26	5	12
Panda #	6.10	2.56	2	12
Panther # ~	9.02	3.97	4	20
Parakeet	9.64	4.58	5	18
Parrot #	6.30	1.72	4	10
Partridge #	8.86	3.57	6	20
Peacock #	7.72	2.91	5	15
Pelican #	5.84	3.89	1	16
Penguin #	5.84	2.56	3	13
Perch	6.68	6.68	1	30
Pheasant	8.21	3.44	5	16
Pig # *	4.56	1.50	3	8
Pigeon	6.00	1.72	4	10
Piglet ~	5.75	2.03	3	10
Pike # ~	9.08	5.57	4	25
Piranha fish # ~	11.49	5.91	7	30
Plaice #	8.25	2.81	5	13
Platypus #	6.79	6.44	1	25
Polar bear	6.95	1.71	5	11
Pony	5.13	2.17	2	10
Porcupine	8.37	2.97	5	15

Animal	Mean	SD	Range min	max
Porpoise	6.87	5.56	1	22
Poultry #	7.52	3.07	4	14
Prairie dog #	12.50	10.06	4	40
Puffin #	8.87	3.99	5	20
Puma #	9.95	3.01	6	16
Rabbit # *	3.98	1.72	2	9
Racoon #	10.04	3.43	5	17
Ram	7.58	3.60	4	15
Rat	5.66	1.86	3	9
Raven ~	8.96	3.31	5	15
Reindeer #	4.30	2.24	2	12
Rhesus monkey	12.76	6.31	5	30
Rhinoceros #	5.46	3.12	1	14
Roach	10.91	6.44	5	30
Robin	5.58	1.53	4	10
Rodent	8.87	2.93	5	15
Rook	7.07	3.44	2	15
Rooster # *	8.10	3.04	5	15
Salamander #	12.11	5.96	5	30
Salmon	7.05	3.09	3	15
Sardine	6.97	3.84	4	17
Sea lion #	8.61	2.39	5	14
Seagull	5.71	2.17	3	10
Seahorse #	7.85	2.93	5	14
Seal #	7.48	2.26	5	12
Shark #	6.96	2.76	4	14
Sheep # *	4.41	1.73	3	8
Shrew	6.59	4.09	1	20
Shrimp	7.14	2.78	4	13
Siamese cat	7.26	3.87	3	15
Siberian tiger #	10.81	6.56	5	30
Skate	6.70	5.83	1	25
Skunk #	6.06	3.84	1	18
Skylark # *	5.65	4.90	1	20
Sloth #	10.05	7.46	4	35
Slug #	5.04	2.04	2	10
Snail	4.81	2.01	2	10
Snake # *	5.89	1.28	4	9
Sole	9.40	2.79	4	16

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Sow #	7.61	4.17	5	20
Sparrow #	5.89	2.03	4	11
Spider	4.02	2.09	3	10
Springbok	7.08	9.14	1	40
Squid #	10.67	3.72	7	20
Squirrel # *	5.99	1.79	4	10
Stag	9.26	3.41	6	20
Star fish #	7.10	3.32	4	15
Starling #	6.40	2.79	3	13
Stick insect #	9.04	4.36	6	24
Stickleback #	5.54	2.71	1	12
Stoat	6.41	4.27	1	20
Sturgeon	7.24	5.50	1	20
Swan	5.46	2.03	3	10
Swift	8.18	3.74	4	20
Sword fish	10.46	6.86	5	30
Tarantula #	10.97	3.51	6	20
Thrush # *	7.31	2.48	5	13
Tiger # *	5.81	1.41	4	9
Toad # *	6.37	1.63	4	10
Tortoise	4.97	2.03	3	10
Trout ~	8.25	3.59	5	20
Tuna #	10.92	5.94	5	30
Turkey # *	5.73	1.76	4	10
Turtle # *	7.15	2.68	5	12
Tyrannosaurus # ~ *	8.83	3.12	5	16
Vole	9.06	5.33	5	25
Vulture # ~	8.40	3.19	5	14
Wallaby #	9.95	3.87	5	20
Walrus	8.49	3.10	5	15
Warthog #	8.65	4.92	2	21
Wasp	4.66	2.33	3	10
Water buffalo #	11.26	5.34	6	25
Water rat	9.14	3.86	5	16
Weasel # *	7.32	2.99	2	13
Whale #	6.35	2.54	4	12
White tiger #	14.61	13.54	5	54
Wild boar #	10.48	4.76	6	22
Wild cat	9.44	7.29	4	35

Animal	Mean	SD	Range min	Range max
Wild dog #	9.70	3.83	5	19
Wildebeest # ~	7.50	5.98	1	23
Wolf	5.81	1.90	4	10
Wombat #	10.17	4.82	3	20
Woodlouse #	7.37	4.10	4	15
Woodpecker	6.90	2.24	4	11
Worm	4.15	1.29	3	8
Wren	6.32	3.84	2	16
Yak #	6.68	6.13	1	25
Yellow tit	11.69	12.79	4	60
Yorkshire terrier *	7.22	3.94	3	20
Zebra # *	6.25	1.61	4	10

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table A.5b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to the 51-60 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	6.37	2.58	3	14
Almond	6.75	2.83	4	12
Apple # *	4.23	1.81	2	8
Apricot #	6.89	4.52	3	20
Aubergine #	15.35	7.90	6	35
Avocado #	13.97	8.43	5	40
Banana # *	4.68	2.15	2	10
Berries	6.05	2.85	3	12
Blackberry	6.04	2.18	3	10
Blackcurrant # *	6.45	2.10	4	10
Blueberry # ~	9.43	15.15	2	55
Bramble ~ *	6.56	2.24	4	11
Cantaloupe melon #	13.85	15.02	4	60
Cherry #	6.67	2.14	4	11
Chestnut	6.65	2.19	4	11
Clementine #	8.34	5.26	5	20
Coconut	6.38	1.98	4	10
Cox apple *	7.28	2.89	4	14
Crab apple	5.23	3.88	1	20
Cranberry #	12.76	11.69	4	50
Cucumber #	5.70	1.99	4	10
Currant	5.60	2.59	4	11
Damson #	8.73	5.17	5	24
Date	6.29	3.36	3	14
Elderberry *	8.03	9.38	2	30
Fig	7.24	5.06	3	20
Gala apple #	16.65	15.55	6	60
Galia melon #	15.43	15.28	5	60
Gooseberry # *	5.21	3.07	2	12
Granny smith #	9.25	4.61	6	20
Grapefruit #	7.82	4.25	5	20
Grape #	6.81	2.63	4	12
Green melon #	12.53	7.40	7	30
Guava # *	16.39	11.70	7	50

Fruit	Mean	SD	Range min	Range max
Hazelnut	6.56	3.71	4	20
Honeydew melon #	12.27	5.92	5	24
Horse chestnut *	7.05	2.65	4	15
Jaffa # *	6.12	2.93	3	13
Kiwi #	14.62	10.09	4	40
Kumquat # *	15.14	13.83	3	60
Lemon #	6.54	2.18	4	10
Lime # *	10.19	5.35	4	25
Loganberry	11.03	15.40	3	60
Lychee #	15.04	11.21	4	40
Mandarin # ~	6.36	3.29	3	15
Mango #	13.51	9.25	5	40
Melon #	8.87	6.24	4	30
Nectarine #	9.78	11.50	4	50
Nut	5.43	1.94	3	10
Olive # ~ *	11.88	9.49	4	40
Orange # *	4.86	1.75	3	10
Papaya #	18.92	10.82	6	40
Passion fruit #	16.85	10.88	6	40
Peach #	6.93	3.10	4	15
Pear #	5.45	2.45	4	12
Pepper # *	13.61	9.06	5	35
Pineapple #	6.95	2.32	4	12
Plum	6.53	3.07	3	15
Pomegranate	6.60	3.07	4	15
Prune	7.63	3.24	3	15
Raisin # ~ *	5.59	2.68	3	11
Raspberry #	7.23	4.12	5	20
Red currant	9.38	12.06	4	40
Redberry	9.20	17.46	4	60
Red grape #	7.93	8.68	4	35
Rhubarb	5.43	2.91	3	15
Satsuma #	7.91	5.56	4	20
Squash #	15.98	13.19	5	45
Star fruit #	17.16	15.59	4	60
Strawberry #	5.49	1.98	3	10
Sultana *	5.75	2.43	3	11
Tangerine #	6.36	3.20	4	15
Tomato #	4.86	2.68	2	12

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range	
			min	max
Walnut	6.48	2.18	4	12
Water melon #	11.52	7.83	4	30
White grape# ~	8.44	5.04	5	20

Table A.6a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to the 61-70 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark #	11.34	10.88	6	50
Adder # ~	9.20	5.76	6	30
Albatross # ~	10.92	3.39	6	16
Alligator # *	6.90	3.28	2	14
Amoeba	11.24	1.29	10	14
Angel fish #	12.27	5.58	7	30
Ant # *	5.40	2.52	2	12
Anteater # ~	9.96	2.61	7	16
Antelope # ~	9.49	3.10	6	16
Ape #	7.38	2.96	4	14
Armadillo #	11.36	3.20	6	18
Ass #	7.40	2.75	3	14
Baboon #	9.79	2.22	7	14
Badger #	7.40	13.04	3	57
Bald eagle #	12.32	10.07	6	50
Bat #	7.33	2.68	4	13
Bear *	4.74	2.29	2	10
Beaver # *	8.41	3.27	5	14
Bee # *	4.24	1.87	2	9
Beetle	4.90	3.11	3	12
Bird	3.15	1.25	2	6
Bison ~	10.18	2.39	7	15
Blackbird	5.78	2.98	3	13
Blue bird #	7.20	3.06	4	14
Blue tit # *	8.17	3.84	5	20
Boa constrictor #	10.30	3.68	7	20
Boar	9.28	2.40	5	13
Brontosaurus # ~	10.48	8.67	5	40
Brown bear #	7.24	3.43	4	16
Buck	10.97	2.47	8	15
Budgerigar #	6.12	2.40	3	12
Buffalo #	8.35	2.86	5	15
Bull	6.17	2.60	3	11
Bullfinch	10.72	3.32	6	20
Bullock	8.50	2.38	5	12

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Butterfly	4.35	2.11	2	12
Buzzard	10.93	2.17	6	16
Calf	5.91	2.53	3	12
Camel	6.31	2.98	3	12
Canary	6.18	2.46	3	12
Caribou	11.75	2.93	8	17
Carp # ~	11.22	5.53	8	30
Cat	3.21	1.15	2	6
Caterpillar	4.66	2.15	3	10
Cattle	6.31	1.99	4	12
Chaffinch	10.27	3.94	6	20
Chameleon #	11.67	3.50	8	18
Cheetah # ~	8.62	2.83	6	15
Chicken # *	3.91	2.19	2	10
Chimpanzee	6.44	2.49	4	12
Chinchilla #	12.46	4.29	9	25
Chipmunk # ~ *	9.84	8.48	4	40
Clown fish #	15.10	14.41	9	50
Cobra # ~	8.81	2.57	4	13
Cockatiel #	10.13	3.50	5	17
Cocker spaniel	8.01	4.34	5	20
Cockerel	5.40	2.33	3	12
Cockroach	8.81	4.30	3	17
Cod	6.94	2.82	5	15
Condor # ~	12.36	7.12	7	30
Conger eel	11.03	5.65	6	30
Cougar # ~	11.44	4.31	7	23
Cow *	3.47	1.46	2	6
Coyote # ~ *	9.58	6.01	4	30
Cray fish # ~	15.46	4.99	10	30
Crocodile #	6.47	2.76	4	12
Crow	6.45	2.19	4	12
Cuckoo	6.02	2.04	4	12
Deer # *	6.05	2.72	3	12
Dingo	11.04	4.27	7	24
Dog	3.01	1.34	2	6
Dolphin #	8.32	2.91	5	15
Donkey	4.16	2.60	2	12
Dormouse	6.73	2.62	3	13

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Dove #	6.85	3.00	4	15
Dragon #	6.14	2.45	4	12
Dragonfly # *	7.69	6.40	3	30
Duck # *	4.10	2.09	2	8
Duckbill platypus #	11.10	2.31	7	15
Eagle	7.66	2.22	4	12
Earthworm	4.14	1.61	2	8
Earwig *	6.32	4.14	4	20
Eel	8.63	2.96	5	16
Elk	9.53	3.71	4	18
Emu #	9.41	2.94	6	15
Ewe	8.39	1.76	6	12
Ferret	8.62	2.90	4	15
Field mouse	6.70	2.74	3	12
Finch	10.13	5.49	5	30
Fish	3.61	1.35	2	6
Flamingo #	9.07	3.94	5	18
Flea	6.51	2.89	4	15
Fly *	3.55	1.89	1	8
Fowl # ~	6.96	2.69	4	13
Fox # *	5.52	3.09	2	14
Frog # *	4.54	2.18	2	9
Gazelle	6.05	3.15	1	14
Gecko #	15.08	7.43	8	30
Gerbil #	10.06	4.84	5	25
Giant panda #	9.68	3.76	5	21
Gibbon #	11.20	2.57	8	16
Giraffe	5.57	2.83	3	13
Goat	5.55	2.74	3	13
Goldfish	4.56	1.75	3	8
Goose #	6.40	3.37	3	14
Gorilla #	6.71	3.15	4	14
Greyhound	7.36	2.09	5	12
Grizzly bear # *	5.64	3.64	1	16
Groundhog # *	12.98	11.82	6	52
Guinea fowl	11.59	3.00	8	20
Guinea pig #	7.09	3.11	4	14
Gull	5.95	2.76	3	12
Haddock	6.78	6.04	4	30

Animal	Mean	SD	Range min	Range max
Hamster #	7.54	3.04	4	15
Hare	6.93	3.03	3	15
Hawk # ~	8.73	2.50	5	14
Hedgehog	5.60	2.12	4	10
Hen	4.37	1.66	2	9
Heron #	8.96	3.06	6	16
Herring	9.62	3.15	7	18
Hippopotamus #	7.25	2.70	4	12
Hornet	10.95	1.72	8	14
Horse	3.56	1.83	2	8
Horsefly	9.70	8.26	7	40
Hyena #	9.34	3.53	4	16
Iguana # *	11.84	8.47	9	40
Insect #	4.70	1.87	2	9
Invertebrate	11.62	2.67	8	17
Jack rabbit	9.30	4.73	4	21
Jackal # ~	10.14	6.07	6	30
Jackass	11.15	2.60	9	17
Jackdaw	8.47	2.80	5	15
Jaguar #	8.78	3.74	6	17
Kangaroo #	6.10	2.51	3	11
Kid	8.21	2.62	5	14
Kitten	3.58	3.13	2	16
Kiwi	11.54	6.15	7	30
Koala #	7.27	3.54	4	14
Koi carp #	16.51	8.72	8	40
Komodo dragon # ~	15.20	5.78	8	30
Lady bird #	5.02	1.97	3	10
Lamb	3.84	1.79	2	8
Lemur #	13.13	5.99	10	30
Leopard #	7.05	2.77	4	12
Lion # *	4.91	2.65	3	12
Lizard #	7.56	4.27	4	20
Llama	10.66	2.67	7	17
Lobster # ~	10.46	5.39	7	30
Long tailed tit	10.00	3.87	6	20
Lynx	11.64	3.65	6	20
Mackerel *	6.50	3.86	1	16
Mammal #	9.51	1.70	7	12

Animal	Mean	SD	Range min	max
Meerkat #	20.68	17.94	7	60
Midge	7.36	2.79	4	12
Mink	10.81	2.60	7	16
Manx	12.17	3.41	6	18
Mole	6.33	2.94	3	14
Mongoose ~	7.96	4.67	4	18
Monkey	4.58	2.29	3	10
Moose # *	8.55	3.10	4	13
Moth	5.35	2.05	3	10
Mouse	4.00	1.91	2	9
Mule	7.56	2.74	3	15
Newt	7.58	2.65	5	14
Nightingale	8.79	2.93	5	15
Octopus #	7.73	6.00	5	30
Orang-utan #	9.52	4.23	6	24
Ostrich #	7.28	3.03	4	13
Otter #	9.06	2.55	5	16
Owl	5.49	2.96	3	15
Ox	7.93	2.35	5	12
Panda #	7.94	3.97	4	17
Panther # ~	9.52	6.65	6	35
Parakeet	8.34	3.71	5	19
Parrot #	6.16	2.90	3	15
Partridge #	9.34	5.84	6	30
Peacock #	7.27	3.98	3	21
Pelican #	8.32	2.70	5	15
Penguin #	6.10	3.27	4	14
Perch	10.81	6.01	7	30
Pheasant	8.94	5.79	6	30
Pig # *	4.01	1.61	2	8
Pigeon	5.86	2.40	3	12
Piglet ~	4.63	2.03	2	10
Pike # ~	10.13	5.78	6	30
Piranha fish # ~	11.08	3.57	7	21
Plaice #	8.41	2.19	6	12
Platypus #	10.16	2.79	8	18
Polar bear	5.77	2.71	4	12
Pony	4.92	1.53	3	8
Porcupine	8.69	3.43	6	15

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Porpoise	7.33	4.15	4	16
Poultry #	6.06	2.22	2	12
Prairie dog #	11.91	7.78	6	30
Puffin #	9.48	3.20	6	15
Puma #	10.01	2.77	6	16
Rabbit # *	4.03	1.71	2	8
Racoon #	10.14	6.16	4	30
Ram	7.26	2.28	4	12
Rat	5.48	2.64	3	11
Raven ~	9.30	3.28	5	17
Reindeer #	4.91	2.37	2	10
Rhesus monkey	13.53	8.06	6	40
Rhinoceros #	7.12	2.41	4	13
Roach	10.67	3.36	6	18
Robin	4.83	1.92	3	8
Rodent	7.66	2.41	5	15
Rook	8.64	2.94	5	15
Rooster # *	6.84	2.64	2	13
Salamander #	12.84	6.61	9	30
Salmon	8.48	2.95	5	16
Sardine	6.75	3.14	4	16
Sea lion #	8.67	2.78	6	16
Seagull	5.14	2.82	3	12
Seahorse #	10.55	3.16	8	18
Seal #	7.69	2.34	4	12
Shark #	7.65	2.61	5	12
Sheep # *	3.97	1.54	2	7
Shrew	10.48	3.67	7	18
Shrimp	7.89	3.71	4	18
Siamese cat	7.83	3.29	5	16
Siberian tiger #	12.82	8.29	5	40
Skate	8.67	3.64	4	16
Skunk #	8.76	2.97	5	14
Skylark # *	9.72	3.50	6	20
Sloth #	10.99	3.66	8	20
Slug #	5.18	2.28	3	12
Snail	4.75	1.84	2	9
Snake # *	5.37	2.36	3	12
Sole	10.64	2.89	6	18

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Sow #	6.79	2.76	4	16
Sparrow #	4.52	2.15	3	11
Spider	3.71	1.79	2	8
Springbok	10.59	3.40	6	17
Squid #	10.96	3.01	8	18
Squirrel # *	5.91	3.22	2	14
Stag	8.37	2.85	6	14
Star fish #	6.98	2.60	4	12
Starling #	7.26	2.92	4	13
Stick insect #	10.52	6.70	6	30
Stickleback #	7.02	3.02	4	15
Stoat	9.56	2.63	5	15
Sturgeon	13.07	5.85	8	30
Swan	5.56	2.28	3	10
Swift	9.25	5.89	6	30
Sword fish	9.75	4.22	5	20
Tarantula #	9.85	4.50	7	25
Thrush # *	7.31	5.00	3	25
Tiger # *	5.06	2.44	3	11
Toad # *	5.36	2.18	2	10
Tortoise	5.26	2.36	3	12
Trout ~	7.62	2.53	4	13
Tuna #	10.47	4.01	4	20
Turkey # *	6.08	2.85	3	12
Turtle # *	6.76	2.74	3	14
Tyrannosaurus # ~ *	10.88	8.56	5	40
Vole	9.22	2.71	6	16
Vulture # ~	9.12	2.66	5	15
Wallaby #	9.61	2.16	6	15
Walrus	8.33	2.60	4	14
Warthog #	11.82	2.31	8	17
Wasp	4.26	1.76	2	8
Water buffalo #	10.54	3.87	6	20
Water rat	7.30	2.96	4	14
Weasel # *	8.28	3.48	3	16
Whale #	5.53	3.28	3	14
White tiger #	12.90	5.05	8	25
Wild boar #	10.57	2.68	8	16
Wild cat	8.45	3.04	4	14

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Wild dog #	9.99	6.10	5	30
Wildebeest # ~	11.67	3.56	8	21
Wolf	5.49	3.36	3	14
Wombat #	12.99	5.65	9	25
Woodlouse #	6.41	4.12	2	16
Woodpecker	7.56	2.91	4	12
Worm	3.94	1.79	2	8
Wren	7.81	2.81	5	15
Yak #	12.35	5.49	7	30
Yellow tit	9.53	3.68	5	17
Yorkshire terrier *	7.46	4.88	3	20
Zebra # *	5.58	3.00	2	13

Table A.6b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to the 61-70 age –category.

Fruit	**Mean**	**SD**	**Range** min	**Range** max
Acorn	5.09	3.45	1	14
Almond	6.43	4.44	2	18
Apple # *	3.67	1.69	2	9
Apricot #	8.16	8.37	3	40
Aubergine #	9.39	13.80	1	55
Avocado #	11.49	9.78	2	40
Banana # *	5.68	2.86	2	12
Berries	4.50	2.20	1	10
Blackberry	6.07	5.89	3	30
Blackcurrant # *	5.15	4.08	1	20
Blueberry # ~	7.18	17.35	1	57
Bramble ~ *	4.78	4.67	1	20
Cantaloupe melon #	7.52	12.04	1	45
Cherry #	5.87	3.68	2	15
Chestnut	5.33	3.19	1	14
Clementine #	6.92	10.59	1	40
Coconut	4.04	3.68	1	12
Cox apple *	6.63	4.36	2	18
Crab apple	5.54	3.19	1	15
Cranberry #	7.77	13.63	1	55
Cucumber #	6.90	4.30	4	20
Currant	6.05	2.54	3	13
Damson #	5.81	5.77	1	25
Date	5.63	3.28	1	14
Elderberry *	6.31	8.36	1	30
Fig	4.98	10.95	1	50
Gala apple #	8.85	16.14	2	50
Galia melon #	11.27	13.78	2	50
Gooseberry # *	5.78	3.94	2	20
Granny smith #	7.61	5.41	4	20
Grapefruit #	5.93	3.70	1	15
Grape #	6.52	3.28	3	15
Green melon #	8.48	9.78	2	40
Guava # *	6.64	18.20	1	50

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	Range max
Hazelnut	6.68	4.67	2	20
Honeydew melon #	12.72	12.28	3	50
Horse chestnut *	5.21	5.41	1	25
Jaffa # *	6.22	6.07	2	25
Kiwi #	12.50	13.85	2	55
Kumquat # *	7.72	17.68	1	55
Lemon #	4.90	3.46	1	14
Lime # *	6.87	8.91	1	35
Loganberry	6.53	10.12	1	40
Lychee #	8.77	15.76	1	55
Mandarin # ~	6.04	4.87	1	20
Mango #	8.35	12.83	1	50
Melon #	8.85	6.70	3	30
Nectarine #	8.68	16.03	4	50
Nut	5.11	1.88	3	10
Olive # ~ *	7.53	11.88	1	50
Orange # *	4.34	2.45	2	11
Papaya #	8.23	13.51	1	50
Passion fruit #	8.35	13.41	1	46
Peach #	7.38	3.85	4	15
Pear #	5.44	3.04	3	12
Pepper # *	13.81	12.44	5	55
Pineapple #	5.39	3.29	1	15
Plum	5.55	2.02	3	10
Pomegranate	5.42	3.27	1	13
Prune	5.40	4.36	1	20
Raisin # ~ *	5.19	2.66	2	12
Raspberry #	4.62	2.85	1	12
Red currant	6.50	11.00	1	50
Redberry	5.30	5.84	1	20
Red grape #	8.51	7.94	4	40
Rhubarb	4.81	2.49	2	10
Satsuma #	8.34	5.86	3	25
Squash #	7.21	14.54	1	50
Star fruit #	8.93	17.94	1	60
Strawberry #	5.68	3.69	3	17
Sultana *	4.90	3.50	1	13
Tangerine #	6.42	3.87	2	15
Tomato #	5.59	2.02	3	10

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range	
			min	max
Walnut	5.51	2.74	2	12
Water melon #	8.89	6.13	3	27
White grape# ~	6.80	3.85	4	16

Table A.7a. Mean, standard deviation (SD) and range (min-max) age of acquisition values for animal words in individuals belonging to > 70 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark #	12.07	7.31	5	30
Adder # ~	7.37	9.59	4	40
Albatross # ~	9.66	3.17	6	16
Alligator # *	6.79	3.11	4	14
Amoeba	9.85	13.68	5	50
Angel fish #	10.87	19.84	4	60
Ant # *	5.81	1.84	4	10
Anteater # ~	8.72	5.13	4	20
Antelope # ~	8.60	7.05	4	30
Ape #	7.09	2.59	4	14
Armadillo #	11.69	6.45	7	30
Ass #	5.48	1.68	3	8
Baboon #	9.75	11.83	6	50
Badger #	7.41	4.23	4	20
Bald eagle #	14.74	13.84	7	50
Bat #	5.29	1.49	3	8
Bear *	4.68	2.43	3	12
Beaver # *	8.91	7.71	4	30
Bee # *	4.18	1.54	2	7
Beetle	4.09	1.54	2	7
Bird	3.12	1.81	2	7
Bison ~	8.50	3.08	4	15
Blackbird	5.26	1.95	3	10
Blue bird #	8.88	5.83	5	26
Blue tit # *	7.30	10.15	4	40
Boa constrictor #	10.30	6.72	7	30
Boar	8.40	3.29	4	15
Brontosaurus # ~	9.42	12.24	6	40
Brown bear #	6.28	2.31	4	10
Buck	8.07	2.62	6	14
Budgerigar #	5.32	3.52	2	17
Buffalo #	7.73	6.75	4	30
Bull	5.55	2.03	4	10
Bullfinch	9.15	4.18	5	17
Bullock	5.98	1.44	4	9

Animal	Mean	SD	Range min	Range max
Butterfly	3.78	1.59	2	7
Buzzard	9.86	9.21	6	40
Calf	4.79	2.27	2	10
Camel	5.87	3.42	4	17
Canary	5.21	2.71	3	12
Caribou	10.89	7.53	5	30
Carp # ~	9.49	16.89	4	60
Cat	3.10	1.73	2	7
Caterpillar	4.04	1.60	2	7
Cattle	5.91	1.85	4	10
Chaffinch	7.02	4.41	4	16
Chameleon #	11.48	6.43	7	30
Cheetah # ~	8.98	13.18	4	50
Chicken # *	3.88	1.79	2	7
Chimpanzee	6.81	2.53	4	12
Chinchilla #	10.08	7.01	7	30
Chipmunk # ~ *	10.25	6.93	7	30
Clown fish #	20.11	27.48	6	70
Cobra # ~	8.69	7.43	4	30
Cockatiel #	9.27	11.32	4	40
Cocker spaniel	6.24	4.01	3	16
Cockerel	4.72	1.87	2	8
Cockroach	5.44	2.66	3	10
Cod	5.75	2.41	2	10
Condor # ~	10.63	9.45	4	30
Conger eel	9.31	4.29	5	17
Cougar # ~	14.85	12.63	7	50
Cow *	3.64	2.26	2	10
Coyote # ~ *	8.69	3.70	5	18
Cray fish # ~	8.30	10.74	3	30
Crocodile #	7.26	2.36	5	12
Crow	5.61	1.70	3	8
Cuckoo	5.73	2.05	4	11
Deer # *	5.74	1.91	3	10
Dingo	9.47	9.63	4	30
Dog	3.01	1.86	2	7
Dolphin #	9.43	6.29	4	30
Donkey	4.13	1.86	3	8
Dormouse	6.00	2.17	4	11

Animal	Mean	SD	Range min	max
Dove #	6.33	4.57	3	18
Dragon #	6.63	5.83	4	25
Dragonfly # *	5.76	1.93	2	10
Duck # *	4.14	1.55	3	7
Duckbill platypus #	11.10	5.21	6	20
Eagle	5.92	5.70	4	25
Earthworm	3.74	2.11	2	9
Earwig *	4.33	1.46	2	7
Eel	6.37	2.14	3	10
Elk	10.15	7.58	6	30
Emu #	8.97	7.47	4	30
Ewe	7.65	3.38	5	16
Ferret	7.66	9.78	4	41
Field mouse	5.46	1.36	4	8
Finch	7.01	4.10	4	15
Fish	3.91	1.70	2	7
Flamingo #	9.00	9.58	4	35
Flea	5.19	2.27	2	12
Fly *	3.41	1.73	2	7
Fowl # ~	5.54	2.28	3	12
Fox # *	4.21	2.16	2	10
Frog # *	4.33	1.80	2	8
Gazelle	10.11	3.51	7	16
Gecko #	10.89	10.95	4	30
Gerbil #	8.53	12.37	4	45
Giant panda #	9.32	7.52	4	30
Gibbon #	10.79	17.52	4	60
Giraffe	5.81	2.44	4	11
Goat	4.64	1.35	3	7
Goldfish	4.06	1.81	2	8
Goose #	4.65	1.48	3	8
Gorilla #	7.35	4.36	4	20
Greyhound	5.99	2.64	4	12
Grizzly bear # *	6.35	2.97	4	15
Groundhog # *	15.13	14.03	7	50
Guinea fowl	10.25	4.65	6	20
Guinea pig #	5.96	5.90	4	26
Gull	4.81	1.71	2	9
Haddock	5.58	2.19	2	10

Animal	Mean	SD	Range min	Range max
Hamster #	7.79	11.72	4	40
Hare	5.68	2.13	3	12
Hawk # ~	8.33	7.87	4	34
Hedgehog	4.89	2.49	3	12
Hen	3.87	1.95	2	8
Heron #	7.77	12.92	4	50
Herring	6.76	4.48	3	18
Hippopotamus #	7.35	2.91	5	15
Hornet	8.72	2.84	6	15
Horse	3.42	1.97	2	7
Horsefly	7.28	2.75	4	12
Hyena #	7.56	3.03	4	14
Iguana # *	12.48	20.71	7	70
Insect #	5.40	23.73	2	74
Invertebrate	9.55	3.94	5	18
Jack rabbit	6.14	3.50	4	14
Jackal # ~	8.56	16.51	4	60
Jackass	8.43	7.14	4	30
Jackdaw	5.91	2.95	3	12
Jaguar #	9.02	6.80	4	30
Kangaroo #	6.16	3.22	4	14
Kid	5.02	2.98	2	12
Kitten	3.89	1.37	3	7
Kiwi	11.92	6.85	7	30
Koala #	7.38	5.66	3	21
Koi carp #	14.33	20.59	4	60
Komodo dragon # ~	17.63	24.11	7	70
Lady bird #	4.55	2.44	3	11
Lamb	4.24	2.53	2	12
Lemur #	11.83	9.50	7	30
Leopard #	6.14	3.45	3	14
Lion # *	4.98	1.79	3	8
Lizard #	7.31	10.60	4	45
Llama	8.49	2.94	4	14
Lobster # ~	8.87	6.79	4	30
Long tailed tit	8.07	3.44	5	15
Lynx	9.93	4.75	5	20
Mackerel *	7.24	6.80	4	30
Mammal #	8.17	1.93	7	12

Animal	Mean	SD	Range min	Range max
Meerkat #	19.60	24.70	7	80
Midge	6.02	2.43	3	10
Mink	7.05	4.16	2	16
Manx	11.86	5.05	6	20
Mole	5.04	2.21	3	9
Mongoose ~	8.44	7.03	4	30
Monkey	4.76	2.46	2	12
Moose # *	8.33	4.81	5	20
Moth	4.85	1.95	3	8
Mouse	4.20	1.49	2	7
Mule	7.02	2.02	4	11
Newt	5.22	2.30	3	11
Nightingale	6.79	2.75	5	12
Octopus #	7.73	3.75	5	19
Orang-utan #	9.36	15.93	4	60
Ostrich #	6.12	4.89	3	20
Otter #	7.73	4.33	5	20
Owl	4.85	1.85	3	8
Ox	6.92	2.02	4	12
Panda #	6.72	5.37	3	20
Panther # ~	8.26	3.31	4	16
Parakeet	8.35	4.15	4	18
Parrot #	5.66	2.40	3	11
Partridge #	6.42	2.30	4	10
Peacock #	6.56	2.59	4	11
Pelican #	7.67	4.78	4	20
Penguin #	5.55	3.35	3	15
Perch	9.48	5.80	6	26
Pheasant	6.01	3.71	4	15
Pig # *	4.17	1.73	3	7
Pigeon	5.12	2.02	3	11
Piglet ~	4.74	2.02	3	10
Pike # ~	8.14	3.89	5	17
Piranha fish # ~	12.83	16.63	7	50
Plaice #	6.48	2.87	2	12
Platypus #	11.17	8.66	7	30
Polar bear	6.10	3.31	4	13
Pony	4.50	2.06	2	8
Porcupine	8.14	6.07	4	28

Animal	Mean	SD	Range min	Range max
Porpoise	7.66	3.84	4	15
Poultry #	5.07	2.22	3	10
Prairie dog #	10.32	7.61	6	30
Puffin #	10.50	5.79	6	26
Puma #	10.77	6.40	6	30
Rabbit # *	4.04	2.52	2	11
Racoon #	8.81	3.65	4	15
Ram	6.37	3.74	3	15
Rat	4.30	1.42	2	7
Raven ~	7.22	2.72	5	12
Reindeer #	3.88	2.03	1	9
Rhesus monkey	8.89	9.71	4	30
Rhinoceros #	6.23	2.99	3	15
Roach	9.12	1.81	7	12
Robin	4.63	3.45	3	16
Rodent	7.57	3.20	4	15
Rook	6.57	1.97	4	10
Rooster # *	5.66	2.26	3	10
Salamander #	12.41	8.95	5	30
Salmon	5.03	8.51	1	35
Sardine	6.56	1.98	4	10
Sea lion #	7.54	2.94	4	14
Seagull	4.44	2.33	2	11
Seahorse #	7.49	10.78	4	40
Seal #	7.01	4.88	4	20
Shark #	7.56	3.15	4	17
Sheep # *	3.87	3.32	2	15
Shrew	8.84	3.41	5	15
Shrimp	6.77	9.81	4	40
Siamese cat	8.76	4.34	6	20
Siberian tiger #	12.40	17.87	5	60
Skate	8.36	4.13	4	18
Skunk #	8.73	3.64	5	16
Skylark # *	5.61	1.92	3	9
Sloth #	12.51	16.60	7	60
Slug #	4.75	2.32	2	11
Snail	4.08	1.90	2	9
Snake # *	4.90	2.72	2	12
Sole	7.80	2.77	4	12

Animal	Mean	SD	Range min	max
Sow #	5.61	2.19	3	10
Sparrow #	4.35	1.64	2	7
Spider	3.76	1.59	2	7
Springbok	10.15	8.51	5	30
Squid #	13.15	5.69	7	30
Squirrel # *	4.84	1.48	3	8
Stag	5.80	2.61	2	13
Star fish #	6.90	21.20	4	70
Starling #	5.52	1.99	3	9
Stick insect #	9.13	12.10	3	45
Stickleback #	5.90	1.40	4	9
Stoat	5.99	2.72	3	12
Sturgeon	10.65	9.83	4	40
Swan	5.21	1.16	4	7
Swift	7.50	13.02	5	50
Sword fish	9.20	10.06	5	40
Tarantula #	8.61	4.52	4	20
Thrush # *	5.75	1.95	3	10
Tiger # *	4.96	1.85	3	10
Toad # *	5.40	2.08	3	11
Tortoise	4.69	2.40	2	11
Trout ~	8.34	4.06	4	20
Tuna #	9.82	15.04	5	50
Turkey # *	5.40	1.70	2	9
Turtle # *	6.71	4.45	4	20
Tyrannosaurus # ~ *	11.70	11.88	6	40
Vole	8.21	3.95	6	20
Vulture # ~	8.97	2.47	6	13
Wallaby #	11.46	15.88	7	60
Walrus	7.46	3.95	5	18
Warthog #	10.02	15.55	4	60
Wasp	4.62	1.41	3	7
Water buffalo #	10.77	15.24	6	50
Water rat	7.70	2.58	4	14
Weasel # *	7.38	3.02	5	16
Whale #	5.78	6.36	3	28
White tiger #	15.93	19.32	7	60
Wild boar #	10.81	9.82	7	38
Wild cat	7.68	8.22	5	32

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Wild dog #	8.68	3.74	6	17
Wildebeest # ~	14.27	20.13	5	60
Wolf	4.78	1.32	3	8
Wombat #	12.50	10.48	5	40
Woodlouse #	6.74	7.57	5	33
Woodpecker	6.60	2.75	4	12
Worm	3.70	1.64	2	7
Wren	7.05	5.90	4	26
Yak #	11.37	7.27	6	30
Yellow tit	8.00	29.96	4	81
Yorkshire terrier *	6.15	4.55	2	20
Zebra # *	5.35	2.35	3	12

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table A.7b. Mean, standard deviation (SD) and range (min-max) age of acquisition values for fruit words in individuals belonging to > 70 age –category.

Fruit	**Mean**	**SD**	**Range** min	max
Acorn	5.62	1.03	4	7
Almond	6.76	1.66	5	10
Apple # *	4.18	1.64	2	7
Apricot #	6.62	3.67	4	15
Aubergine #	16.13	22.87	7	71
Avocado #	19.28	21.00	7	65
Banana # *	4.88	4.39	3	20
Berries	5.20	1.43	3	7
Blackberry	5.03	2.10	3	10
Blackcurrant # *	5.68	0.94	4	7
Blueberry # ~	12.66	21.47	7	70
Bramble ~ *	6.26	3.94	4	16
Cantaloupe melon #	15.88	22.78	6	65
Cherry #	6.13	7.29	4	30
Chestnut	6.61	1.64	5	10
Clementine #	11.91	20.76	7	65
Coconut	6.73	2.26	4	12
Cox apple *	8.01	9.41	6	40
Crab apple	7.17	2.66	4	14
Cranberry #	18.59	25.00	6	70
Cucumber #	6.84	4.01	4	17
Currant	5.82	1.47	4	8
Damson #	5.64	1.48	4	8
Date	6.37	2.63	5	14
Elderberry *	5.99	1.78	4	10
Fig	6.85	4.52	4	18
Gala apple #	18.97	25.33	7	78
Galia melon #	19.13	25.24	5	71
Gooseberry # *	5.41	2.27	4	12
Granny smith #	9.53	13.94	5	40
Grapefruit #	8.09	3.38	6	17
Grape #	5.40	2.48	3	12
Green melon #	10.81	9.42	7	30
Guava # *	16.73	14.34	7	50

Fruit	Mean	SD	Range min	Range max
Hazelnut	6.06	6.90	2	30
Honeydew melon #	14.41	19.16	7	64
Horse chestnut *	6.53	1.47	4	10
Jaffa # *	7.44	1.92	5	10
Kiwi #	21.88	19.87	7	60
Kumquat # *	18.60	26.96	5	80
Lemon #	5.86	7.55	3	30
Lime # *	10.73	7.03	7	30
Loganberry	6.54	8.91	3	30
Lychee #	18.62	18.22	7	60
Mandarin # ~	10.68	12.46	5	47
Mango #	18.11	12.45	7	50
Melon #	7.96	6.24	4	20
Nectarine #	13.68	20.90	7	53
Nut	5.13	2.06	3	11
Olive # ~ *	10.21	13.80	5	50
Orange # *	4.35	1.99	2	8
Papaya #	16.07	26.40	7	70
Passion fruit #	18.89	20.68	7	70
Peach #	6.67	4.41	3	20
Pear #	4.72	2.18	3	10
Pepper # *	20.15	17.97	7	60
Pineapple #	9.15	10.27	4	40
Plum	5.44	6.65	3	28
Pomegranate	8.78	7.58	5	30
Prune	5.71	3.11	4	15
Raisin # ~ *	6.13	2.78	4	14
Raspberry #	5.17	1.37	3	7
Red currant	6.33	2.12	4	10
Redberry	9.41	53.03	5	80
Red grape #	7.91	3.02	4	15
Rhubarb	5.20	1.56	3	8
Satsuma #	12.35	17.91	5	60
Squash #	13.13	12.50	7	40
Star fruit #	22.95	28.41	7	80
Strawberry #	5.42	3.28	3	16
Sultana *	5.85	2.01	4	10
Tangerine #	8.22	6.82	5	30
Tomato #	4.84	4.11	3	19

~ significant effect of age, **#** significant effect of gender, ***** significant effect of education

Fruit	Mean	SD	Range min	max
Walnut	7.01	3.55	5	18
Water melon #	10.26	19.15	5	70
White grape# ~	7.61	12.90	4	40

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

APPENDIX B

Table B.1a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to the 18-20 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	1.70	1.76	1	6
Adder	2.25	1.99	1	7
Albatross	1.60	1.84	1	7
Alligator ~	3.16	2.02	1	7
Alpaca	1.51	1.61	1	6
Amoeba	1.29	1.59	1	7
Angel fish	1.60	1.65	1	7
Ant	2.88	2.34	1	7
Ant bear	1.17	1.26	1	6
Anteater	1.97	1.98	1	7
Antelope	2.00	1.97	1	7
Ape	3.54	2.06	1	7
Armadillo	1.83	1.48	1	7
Ass	1.85	1.92	1	7
Baboon ~	3.05	2.02	1	7
Badger	2.94	2.07	1	7
Bald eagle #	1.98	2.00	1	7
Bat	3.88	1.75	2	7
Bear	3.17	2.31	1	7
Beaver	2.99	1.75	1	7
Bee	4.97	1.52	2	7
Beetle	3.36	2.16	1	7
Bird	4.85	1.56	1	7
Bison #	1.48	1.60	1	6
Blackbird ~	3.41	1.96	1	7
Blue bird	2.52	1.79	1	7
Blue tit ~	2.61	1.53	1	7
Boa constrictor	2.25	2.01	1	7
Boar	2.02	2.02	1	7
Brontosaurus #	1.43	1.87	1	7
Brown bear	2.81	1.97	1	7
Buck # ~ *	1.51	1.30	1	5
Budgerigar ~	1.87	2.11	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Buffalo	2.22	1.88	1	7
Bull	3.59	1.83	1	7
Bullfinch ~	1.24	1.08	1	5
Bullock ~	1.82	1.83	1	7
Butterfly	4.34	1.72	1	7
Buzzard # *	1.44	1.75	1	6
Calf	2.68	2.26	1	7
Camel	3.15	1.95	1	7
Canary	2.64	1.79	1	7
Caribou	1.35	1.11	1	5
Carp	2.32	1.82	1	7
Cat	5.98	1.19	3	7
Caterpillar	3.69	1.84	2	7
Cattle	4.75	1.72	2	7
Chaffinch ~	1.64	1.47	1	6
Chameleon ~	2.05	2.09	1	7
Cheetah ~	3.68	1.79	1	7
Chicken	4.87	1.51	1	7
Chimpanzee	3.10	2.29	1	7
Chinchilla	2.73	1.82	1	7
Chipmunk	2.31	2.13	1	7
Civet	1.06	0.68	1	4
Clown fish ~	2.07	1.96	1	7
Cobra	2.46	2.20	1	7
Cockatiel	1.84	1.80	1	7
Cocker spaniel	2.68	2.21	1	7
Cockerel	2.72	1.98	1	7
Cockroach	2.61	1.96	1	7
Cod	3.24	2.04	1	7
Condor	1.31	1.19	1	5
Conger eel	1.31	0.88	1	4
Cougar #	1.83	1.40	1	6
Cow	4.73	1.68	1	7
Coyote ~ *	1.78	1.80	1	7
Cray fish	1.70	1.81	1	7
Crocodile	3.13	2.04	1	7
Crow	3.16	1.98	1	7
Cuckoo	2.28	1.89	1	7
Curlew ~	1.13	1.31	1	6

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Deer	3.80	1.92	1	7
Dingo	1.66	1.63	1	7
Dog	6.46	0.84	3	7
Dolphin ~	4.45	1.73	1	7
Donkey	3.82	2.03	1	7
Dormouse	2.16	1.73	1	7
Dove	3.84	1.56	2	7
Dragon ~	2.09	2.49	1	7
Dragonfly	2.58	2.13	1	7
Dromedary ~	1.08	0.67	1	4
Duck	4.87	1.56	1	7
Duckbill platypus	1.43	1.92	1	7
Dugong	1.23	1.57	1	7
Duiker	1.12	0.55	1	3
Eagle	2.79	2.12	1	7
Earthworm	3.28	1.93	1	7
Earwig	2.15	1.67	1	7
Echidna	1.29	1.31	1	5
Eel	2.25	2.01	1	7
Elephant	4.15	2.05	1	7
Elk ~	1.40	1.51	1	6
Emu	2.00	2.12	1	7
Ewe ~	2.34	2.06	1	7
Ferret	2.66	2.01	1	7
Field mouse	2.86	1.98	1	7
Finch ~	2.33	1.64	1	7
Fish ~	5.92	1.26	2	7
Flamingo	2.83	1.97	1	7
Flea	2.44	2.13	1	7
Fly	4.14	1.80	1	7
Fowl	1.98	1.96	1	7
Fox	4.38	1.64	1	7
Frog	3.71	1.76	1	7
Gazelle	2.15	1.65	1	6
Gecko	1.72	1.87	1	7
Gerbil	3.03	2.01	1	7
Giant panda	2.25	2.04	1	7
Gibbon	1.87	2.00	1	7
Giraffe	3.26	2.18	1	7

Animal	Mean	SD	Range min	Range max
Gnu	1.13	0.99	1	5
Goat	3.62	1.94	1	7
Goldcrest	1.39	1.48	1	7
Goldfish	4.56	1.88	2	7
Goose	3.67	1.63	1	7
Gorilla	3.75	2.01	1	7
Greyhound	3.53	1.96	1	7
Grizzly bear	2.88	1.99	1	7
Groundhog #	1.51	1.77	1	7
Guinea fowl	1.87	1.61	1	6
Guinea pig	3.52	1.92	1	7
Gull ~	2.17	2.24	1	7
Haddock	2.27	2.05	1	7
Hamster ~	4.44	1.77	1	7
Hare	2.56	1.96	1	7
Hart	1.32	1.12	1	5
Hawk	3.14	1.78	1	7
Hedgehog	3.81	1.86	1	7
Heifer #	1.19	0.99	1	5
Hen	3.98	1.88	1	7
Heron	2.28	1.91	1	6
Herring	2.10	1.74	1	7
Hippopotamus	3.03	2.24	1	7
Hornet	2.28	2.11	1	7
Horse	4.42	1.81	1	7
Horsefly	1.50	2.01	1	7
Hyena	2.15	2.13	1	7
Ibex	1.21	1.18	1	6
Iguana	1.80	2.30	1	7
Impala	1.26	1.85	1	7
Insect	4.87	1.77	2	7
Invertebrate # ~ *	2.75	2.40	1	7
Jack rabbit # *	1.83	1.50	1	5
Jackal	1.67	1.92	1	7
Jackass	1.37	1.11	1	5
Jackdaw ~	1.22	1.19	1	5
Jaguar	2.56	2.04	1	7
Jerboa	1.08	0.50	1	3
Kangaroo	3.22	2.08	1	7

Animal	Mean	SD	Range min	max
Kid	1.70	2.30	1	7
Kitten	4.38	1.75	1	7
Kiwi	1.66	1.86	1	7
Koala	2.38	2.24	1	7
Koi carp	1.68	1.81	1	7
Komodo dragon # *	1.56	2.29	1	7
Lacewing	1.25	0.97	1	4
Lady bird	4.27	1.87	2	7
Lamb	4.08	1.78	1	7
Lemur	1.78	2.17	1	7
Leopard	3.15	2.12	1	7
Lion	4.42	1.93	2	7
Lizard	2.69	2.11	1	7
Llama	2.46	2.04	1	7
Lobster	3.66	2.06	1	7
Long tailed tit	1.46	1.12	1	5
Lynx	1.52	1.47	1	6
Mackerel	2.31	1.93	1	7
Mammal ~	3.81	1.93	1	7
Manatee	1.53	1.83	1	7
Mandrill ~ *	1.29	1.36	1	6
Mangust	1.08	0.36	1	2
Marmoset *	1.26	1.55	1	7
Marmot	1.17	0.68	1	3
Marten	1.44	1.44	1	6
Meerkat	2.36	2.26	1	7
Midge	1.83	2.05	1	7
Mink *	1.44	1.45	1	5
Minnow # *	1.75	1.35	1	5
Manx	1.99	2.08	1	7
Mole	2.81	1.79	1	7
Mongoose #	1.66	1.66	1	7
Monkey ~	5.55	1.40	2	7
Moose ~	2.61	2.08	1	7
Moth	3.36	1.91	1	7
Mouse	4.27	1.85	1	7
Mule	1.83	2.19	1	7
Musk ox ~ *	1.15	0.63	1	3
Newt	1.76	1.83	1	6

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Nightingale	1.57	1.50	1	6
Ocelot	1.16	1.24	1	6
Octopus ~	3.17	2.09	1	7
Orang-utan	2.60	2.28	1	7
Oryx	1.14	0.76	1	4
Ostrich	2.64	2.07	1	7
Otter	2.44	2.11	1	7
Owl	3.21	1.95	1	7
Ox	2.11	1.91	1	7
Panda	3.22	2.06	1	7
Panther	3.15	1.93	1	7
Parakeet	1.75	1.55	1	6
Parrot	3.81	1.87	1	7
Partridge	2.11	1.69	1	6
Peacock	3.00	2.01	1	7
Peewit ~	1.14	1.27	1	5
Pelican	2.17	1.77	1	7
Penguin	3.82	1.93	1	7
Perch	1.60	1.47	1	6
Pheasant	2.97	1.81	1	7
Pig	4.66	1.75	1	7
Pigeon	4.87	1.57	1	7
Piglet	3.15	2.20	1	7
Pike #	2.18	1.96	1	7
Pine marten	1.36	1.40	1	5
Piranha fish #	2.45	1.86	1	7
Plaice	2.07	1.80	1	7
Platypus	1.57	2.32	1	7
Polar bear	3.23	2.05	1	7
Polar cat	1.48	1.52	1	6
Pony	4.01	1.95	1	7
Porcupine	1.95	1.93	1	7
Porpoise *	1.53	1.71	1	6
Poultry	3.22	2.03	1	7
Prairie dog	1.82	1.80	1	7
Puffin	2.16	1.70	1	7
Puma	2.65	2.02	1	7
Rabbit	5.02	1.68	2	7
Racoon *	2.36	1.95	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Ram	2.41	2.03	1	7
Rat	4.53	1.69	1	7
Ratel	1.12	1.33	1	7
Raven	2.46	1.97	1	7
Reindeer	3.67	2.06	1	7
Rhesus monkey	1.88	2.06	1	7
Rhinoceros	3.03	2.16	1	7
Roach	1.90	1.72	1	6
Robin	4.67	1.56	2	7
Rodent	3.56	1.90	1	7
Roe deer ~	1.19	1.21	1	5
Rook # ~	1.68	1.68	1	7
Rooster	2.88	2.04	1	7
Salamander	1.52	1.59	1	6
Salmon	3.81	1.88	1	7
Sardine	2.67	1.57	1	7
Sea lion	2.22	2.19	1	7
Seagull	4.22	1.69	1	7
Seahorse	2.71	1.88	1	7
Seal	3.53	1.97	1	7
Shark ~	4.07	1.82	1	7
Sheep	4.69	1.77	1	7
Short tailed tit	1.59	1.37	1	5
Shrew	1.88	1.82	1	7
Shrimp	2.92	1.91	1	7
Siamese cat	2.67	2.21	1	7
Siberian tiger ~	2.15	2.36	1	7
Skate	1.44	1.07	1	4
Skunk	2.30	2.25	1	7
Skylark ~	1.33	1.31	1	6
Sloth # ~	2.40	2.21	1	7
Slug	3.10	2.17	1	7
Snail	3.62	1.95	1	7
Snake ~	4.08	1.89	1	7
Sole ~	1.34	1.65	1	7
Sow ~	1.79	1.90	1	6
Sparrow ~	2.86	2.18	1	7
Spider	5.22	1.51	2	7
Springbok	1.74	1.65	1	6

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Squid	2.58	1.92	1	7
Squirrel	4.34	1.78	1	7
Stag	2.61	2.17	1	7
Star fish	2.30	2.28	1	7
Starling ~	1.95	1.96	1	7
Stick insect	2.64	2.18	1	7
Stickleback	1.79	1.17	1	5
Stoat	1.65	1.68	1	7
Sturgeon	1.43	1.17	1	5
Swan	3.76	1.88	1	7
Swift ~	1.50	1.52	1	6
Sword fish ~	2.67	1.86	1	7
Tapir	1.29	1.04	1	4
Tarantula ~ *	2.89	2.19	1	7
Tench	1.26	1.17	1	5
Thrush ~	2.09	1.88	1	6
Tiger	3.47	2.18	1	7
Toad	3.11	1.96	1	7
Tortoise	3.46	1.76	1	7
Trout	2.84	1.93	1	7
Tuna	3.92	1.85	1	7
Turkey	5.54	1.16	3	7
Turtle ~	3.64	1.90	1	7
Tyrannosaurus ~	1.96	2.43	1	7
Vole	1.87	1.83	1	7
Vulture	2.23	2.11	1	7
Wallaby	1.57	1.89	1	7
Walrus	1.92	1.93	1	7
Warthog	1.86	1.83	1	7
Wasp	4.21	1.75	1	7
Water buffalo #	1.94	1.61	1	6
Water rat #	1.79	1.86	1	7
Weasel	2.02	1.91	1	7
Whale ~	3.30	2.20	1	7
White tiger	2.14	2.16	1	7
Wild boar	2.17	1.73	1	7
Wild cat	2.59	1.84	1	7
Wild dog	1.91	1.48	1	6
Wildebeest	1.98	1.46	1	6

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Wolf ~	3.16	2.17	1	7
Wombat	1.76	1.80	1	7
Woodcock	1.46	1.51	1	7
Woodlouse	3.49	2.03	1	7
Woodpecker	2.56	1.90	1	7
Worm	3.55	2.05	1	7
Wren ~	1.71	1.69	1	6
Yak	1.57	1.33	1	5
Yellow tit	1.51	1.42	1	5
Yellowhammer ~	1.31	1.42	1	5
Yorkshire terrier	4.47	1.62	1	7
Zebra	2.85	2.29	1	7
Zebu	1.10	1.14	1	6

Table B.1b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to the 18-20 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	2.78	2.06	1	7
Almond	3.01	1.85	1	7
Apple	6.46	0.84	3	7
Apricot	4.08	1.59	1	7
Aubergine	2.26	1.85	1	7
Avocado	2.75	1.90	1	7
Banana	6.31	0.98	2	7
Berries	5.09	1.43	2	7
Bilberry #	1.26	1.32	1	7
Blackberry	4.10	1.65	1	7
Blackcurrant	5.13	1.33	1	7
Blueberry	3.80	1.97	1	7
Bramble #	1.74	2.05	1	7
Butternut squash	2.51	2.09	1	7
Cantaloupe melon	2.30	2.04	1	7
Cherry	5.09	1.39	2	7
Chestnut	3.15	1.86	1	7
Citron	1.81	2.23	1	7
Clementine # ~	3.94	1.90	1	7
Coconut	3.44	2.07	1	7
Cox apple #	2.97	2.20	1	7
Crab apple	2.10	2.15	1	7
Cranberry	3.90	1.71	1	7
Cucumber	4.52	1.76	1	7
Currant	2.99	1.94	1	7
Damson #	1.21	0.91	1	4
Date #	1.85	2.00	1	7
Dewberry	1.47	1.57	1	7
Durian	1.13	0.95	1	5
Elderberry #	1.77	1.75	1	7
Fig	2.02	2.03	1	7
Gala apple	3.35	2.04	1	7
Galia melon ~	2.21	2.15	1	7
Gooseberry	2.51	2.03	1	7
Granny smith	5.94	1.11	3	7
Grapefruit	4.07	1.72	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	max
Grapes	5.32	1.37	1	7
Green melon	3.01	1.84	1	7
Guava	1.53	1.55	1	7
Haw #	1.02	0.22	1	2
Hazelnut	2.79	1.98	1	7
Honeydew melon ~	2.71	2.12	1	7
Horse chestnut	2.03	1.71	1	6
Jackfruit	1.17	0.78	1	4
Jaffa #	1.48	1.79	1	7
Kiwi ~	4.30	1.70	1	7
Kumquat	1.31	1.09	1	5
Lemon	5.24	1.45	2	7
Lime #	3.80	1.90	1	7
Loganberry #	1.35	1.31	1	6
Lychee	1.84	2.06	1	7
Mandarin ~	2.72	2.15	1	7
Mango #	4.56	1.48	1	7
Medlar	1.09	0.70	1	4
Melon	4.31	1.84	1	7
Nectarine ~	3.48	1.97	1	7
Nuts	3.83	1.94	1	7
Olive	3.53	1.96	1	7
Orange	6.35	0.93	3	7
Papaya	1.83	2.06	1	7
Passion fruit	3.18	1.94	1	7
Paw paw	1.21	1.64	1	7
Peach #	5.34	1.33	2	7
Pear	5.70	1.15	3	7
Pepper	5.34	1.28	2	7
Persimmon	1.09	0.52	1	3
Pineapple	5.43	1.46	2	7
Plantain	1.32	1.72	1	6
Plum	4.11	1.79	1	7
Pomelo	1.15	0.45	1	2
Pomegranate	2.82	1.84	1	7
Prune #	2.04	2.06	1	7
Quince #	1.08	0.37	1	2
Raisin	5.02	1.50	2	7
Rambotan	1.07	0.50	1	3

Fruit	Mean	SD	Range min	Range max
Raspberry	5.31	1.43	3	7
Red currant	2.61	2.05	1	7
Redberry #	2.82	2.00	1	7
Red grape	3.96	1.88	1	7
Rhubarb	3.29	1.91	1	7
Rosehip #	1.19	1.32	1	5
Sapodilla	1.05	0.32	1	2
Satsuma ~	5.06	1.47	2	7
Sharon fruit	1.20	1.75	1	7
Sloe berry	1.14	0.44	1	2
Squash	2.14	2.06	1	7
Star fruit	1.69	1.83	1	7
Strawberry	6.04	1.09	2	7
Sultana	4.25	1.74	1	7
Tangelo *	1.12	0.93	1	5
Tangerine	5.28	1.48	2	7
Tayberry	1.33	1.42	1	6
Tomato	6.31	0.93	3	7
Walnut #	2.95	1.84	1	7
Water melon	4.49	1.76	1	7
Whinberry	1.38	1.25	1	6
White currant	1.55	1.79	1	7
White grape	3.44	2.08	1	7
Whortleberry	1.22	1.12	1	6

Table B.2a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to the 21-30 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	1.91	2.07	1	7
Adder	2.55	2.17	1	7
Albatross	2.23	2.05	1	7
Alligator ~	3.68	1.94	1	7
Alpaca	1.23	1.37	1	5
Amoeba	1.88	2.28	1	7
Angel fish	1.72	2.24	1	7
Ant	3.92	2.08	1	7
Ant bear	1.50	1.53	1	7
Anteater	2.12	1.95	1	7
Antelope	2.22	2.08	1	7
Ape	4.39	1.77	1	7
Armadillo	2.30	2.00	1	7
Ass	2.40	1.98	1	7
Baboon ~	3.23	2.00	1	7
Badger	3.73	1.88	1	7
Bald eagle #	2.74	1.98	1	7
Bat	3.10	2.16	1	7
Bear	4.65	1.65	1	7
Beaver	2.86	2.12	1	7
Bee	4.85	1.81	2	7
Beetle	3.44	2.08	1	7
Bird	5.32	1.46	1	7
Bison #	2.06	2.02	1	7
Blackbird ~	3.95	2.01	1	7
Blue bird	2.49	2.26	1	7
Blue tit ~	2.58	2.15	1	7
Boa constrictor	2.78	2.17	1	7
Boar	3.10	2.08	1	7
Brontosaurus #	1.97	2.20	1	7
Brown bear	3.18	2.03	1	7
Buck # ~ *	1.69	1.99	1	7
Budgerigar ~	2.75	2.05	1	7
Buffalo	3.18	1.97	1	7
Bull	3.41	2.12	1	7
Bullfinch ~	1.60	1.82	1	7

Animal	Mean	SD	Range min	Range max
Bullock ~	2.09	2.31	1	7
Butterfly	4.09	1.91	1	7
Buzzard # *	1.98	2.12	1	7
Calf	4.60	1.71	2	7
Camel	2.67	2.52	1	7
Canary	2.68	2.02	1	7
Caribou	1.53	1.79	1	6
Carp	2.60	2.04	1	7
Cat	5.57	1.29	1	7
Caterpillar	3.38	2.15	1	7
Cattle	5.44	1.43	2	7
Chaffinch ~	2.02	2.13	1	7
Chameleon ~	2.75	2.08	1	7
Cheetah ~	3.34	2.10	1	7
Chicken	5.31	1.41	1	7
Chimpanzee	3.93	1.85	1	7
Chinchilla	2.33	1.80	1	7
Chipmunk	2.39	2.02	1	7
Civet	1.18	1.53	1	6
Clown fish ~	2.30	2.29	1	7
Cobra	2.54	2.20	1	7
Cockatiel	2.46	2.14	1	7
Cocker spaniel	4.45	1.68	1	7
Cockerel	3.59	1.78	1	7
Cockroach	2.95	2.12	1	7
Cod	3.81	1.76	1	7
Condor	1.89	1.88	1	7
Conger eel	1.59	1.85	1	7
Cougar #	1.87	2.19	1	7
Cow	4.74	1.64	1	7
Coyote ~ *	2.72	1.94	1	7
Cray fish	1.97	2.11	1	7
Crocodile	3.89	1.97	1	7
Crow	3.72	2.08	1	7
Cuckoo	2.46	2.02	1	7
Curlew ~	1.16	1.60	1	7
Deer	3.99	1.88	1	7
Dingo	1.78	2.08	1	7
Dog	5.85	1.07	1	7

Animal	Mean	SD	Range min	max
Dolphin ~	3.85	2.10	1	7
Donkey	4.70	1.65	1	7
Dormouse	2.42	2.26	1	7
Dove	3.37	2.09	1	7
Dragon ~	2.58	2.45	1	7
Dragonfly	2.28	2.42	1	7
Dromedary ~	1.28	2.33	1	7
Duck	4.58	1.74	1	7
Duckbill platypus	1.69	1.94	1	7
Dugong	1.28	2.06	1	7
Duiker	1.31	2.31	1	7
Eagle	2.95	2.25	1	7
Earthworm	2.91	2.32	1	7
Earwig	2.81	2.19	1	7
Echidna	1.27	2.02	1	7
Eel	2.42	2.21	1	7
Elephant	4.99	1.71	2	7
Elk ~	1.65	2.04	1	7
Emu	2.27	2.17	1	7
Ewe ~	1.84	2.32	1	7
Ferret	3.25	2.05	1	7
Field mouse	2.82	2.32	1	7
Finch ~	2.11	1.97	1	7
Fish ~	6.25	1.00	2	7
Flamingo	3.28	1.96	1	7
Flea	3.48	2.08	1	7
Fly	4.67	1.82	1	7
Fowl	2.59	2.26	1	7
Fox	4.09	1.95	1	7
Frog	4.10	2.06	1	7
Gazelle	1.96	2.28	1	7
Gecko	2.27	2.29	1	7
Gerbil	3.42	1.97	1	7
Giant panda	2.55	2.42	1	7
Gibbon	2.10	2.10	1	7
Giraffe	3.55	2.12	1	7
Gnu	1.34	1.96	1	7
Goat	4.01	1.95	1	7
Goldcrest	1.39	1.64	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Goldfish	5.29	1.53	2	7
Goose	4.38	1.82	1	7
Gorilla	4.71	1.73	1	7
Greyhound	4.64	1.59	2	7
Grizzly bear	3.11	2.04	1	7
Groundhog #	1.55	1.55	1	6
Guinea fowl	1.99	2.21	1	7
Guinea pig	3.97	1.99	1	7
Gull ~	3.25	2.06	1	7
Haddock	3.91	1.70	1	7
Hamster ~	5.08	1.55	2	7
Hare	3.03	2.06	1	7
Hart	1.43	1.72	1	7
Hawk	2.49	2.21	1	7
Hedgehog	4.31	1.92	1	7
Heifer #	1.54	2.20	1	7
Hen	4.43	1.79	1	7
Heron	2.30	2.11	1	7
Herring	2.38	2.12	1	7
Hippopotamus	3.26	2.20	1	7
Hornet	2.32	2.09	1	7
Horse	5.58	1.47	2	7
Horsefly	2.00	2.24	1	7
Hyena	2.35	2.27	1	7
Ibex	1.23	1.42	1	7
Iguana	2.56	1.99	1	7
Impala	1.35	1.32	1	6
Insect	5.96	1.17	3	7
Invertebrate # ~ *	2.66	2.36	1	7
Jack rabbit # *	1.99	2.00	1	7
Jackal	1.84	2.00	1	7
Jackass	1.65	1.87	1	7
Jackdaw ~	1.45	1.96	1	7
Jaguar	2.55	2.12	1	7
Jerboa	1.26	1.79	1	7
Kangaroo	3.31	2.20	1	7
Kid	2.37	2.26	1	7
Kitten	4.72	1.75	1	7
Kiwi	1.83	2.29	1	7

Animal	Mean	SD	Range min	Range max
Koala	3.07	2.18	1	7
Koi carp	2.36	2.35	1	7
Komodo dragon # *	2.23	2.17	1	7
Lacewing	1.30	1.62	1	7
Lady bird	4.34	1.87	1	7
Lamb	4.52	1.78	1	7
Lemur	1.93	1.58	1	6
Leopard	4.01	2.01	2	7
Lion	4.78	1.81	2	7
Lizard	3.78	1.87	1	7
Llama	2.61	1.93	1	7
Lobster	3.35	2.25	1	7
Long tailed tit	1.65	1.77	1	7
Lynx	1.83	1.98	1	7
Mackerel	2.88	1.95	1	7
Mammal ~	5.82	1.23	3	7
Manatee	1.69	2.02	1	7
Mandrill ~ *	1.40	1.85	1	7
Mangust	1.15	1.82	1	7
Marmoset *	1.25	1.67	1	7
Marmot	1.41	1.67	1	7
Marten	1.47	1.87	1	7
Meerkat	2.60	2.17	1	7
Midge	2.16	2.24	1	7
Mink *	1.60	1.78	1	6
Minnow # *	1.58	1.49	1	6
Manx	2.01	2.22	1	7
Mole	3.21	1.85	1	7
Mongoose #	2.04	1.94	1	7
Monkey ~	5.01	1.54	1	7
Moose ~	2.91	2.03	1	7
Moth	3.83	1.96	1	7
Mouse	3.75	2.09	1	7
Mule	2.32	2.11	1	7
Musk ox ~ *	1.10	0.78	1	4
Newt	2.53	2.11	1	7
Nightingale	1.86	1.81	1	7
Ocelot	1.38	2.24	1	7
Octopus ~	3.47	2.05	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Orang-utan	3.36	2.03	1	7
Oryx	1.16	1.18	1	5
Ostrich	2.65	2.23	1	7
Otter	2.78	2.38	1	7
Owl	4.17	1.93	1	7
Ox	2.47	1.95	1	7
Panda	3.87	1.98	1	7
Panther	3.18	2.19	1	7
Parakeet	2.15	1.93	1	7
Parrot	3.53	2.16	1	7
Partridge	2.55	2.10	1	7
Peacock	3.25	2.13	1	7
Peewit ~	1.19	1.46	1	6
Pelican	2.53	1.93	1	7
Penguin	4.10	1.79	1	7
Perch	1.86	1.58	1	7
Pheasant	3.55	2.03	1	7
Pig	4.95	1.68	1	7
Pigeon	4.86	1.69	1	7
Piglet	3.70	2.09	1	7
Pike #	2.40	1.98	1	7
Pine marten	1.36	1.48	1	5
Piranha fish #	2.57	2.06	1	7
Plaice	2.26	2.08	1	7
Platypus	1.95	2.13	1	7
Polar bear	3.69	2.01	1	7
Polar cat	1.64	2.02	1	7
Pony	4.39	1.73	1	7
Porcupine	1.84	2.18	1	7
Porpoise *	1.70	2.06	1	7
Poultry	4.33	1.71	1	7
Prairie dog	1.87	1.86	1	6
Puffin	2.63	2.25	1	7
Puma	2.50	2.29	1	7
Rabbit	5.32	1.55	2	7
Racoon *	2.32	2.32	1	7
Ram	2.35	2.07	1	7
Rat	3.28	2.28	1	7
Ratel	1.17	1.35	1	5

Animal	Mean	SD	Range min	max
Raven	3.15	1.92	1	7
Reindeer	3.83	2.02	1	7
Rhesus monkey	2.19	2.01	1	7
Rhinoceros	3.49	2.06	1	7
Roach	2.23	1.93	1	7
Robin	4.37	1.73	1	7
Rodent	3.86	2.09	1	7
Roe deer ~	1.63	2.08	1	7
Rook # ~	1.97	2.10	1	7
Rooster	2.90	2.17	1	7
Salamander	1.97	1.85	1	7
Salmon	4.27	1.63	1	7
Sardine	2.97	1.93	1	7
Sea lion	2.91	2.22	1	7
Seagull	4.65	1.76	2	7
Seahorse	2.59	2.05	1	7
Seal	3.82	2.00	1	7
Shark ~	4.93	1.72	2	7
Sheep	4.76	1.79	1	7
Short tailed tit	1.75	1.63	1	7
Shrew	2.37	2.08	1	7
Shrimp	2.95	2.07	1	7
Siamese cat	3.33	1.97	1	7
Siberian tiger ~	2.22	2.38	1	7
Skate	1.89	1.94	1	7
Skunk	2.25	2.34	1	7
Skylark ~	1.66	1.66	1	7
Sloth # ~	2.05	2.36	1	7
Slug	3.56	2.20	1	7
Snail	3.64	2.19	1	7
Snake ~	3.64	2.23	1	7
Sole ~	1.83	1.80	1	7
Sow ~	2.54	2.24	1	7
Sparrow ~	3.70	1.87	1	7
Spider	4.50	1.76	1	7
Springbok	1.71	2.30	1	7
Squid	3.24	1.87	1	7
Squirrel	5.03	1.71	2	7
Stag	2.82	2.13	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Star fish	2.89	2.27	1	7
Starling ~	2.16	2.25	1	7
Stick insect	2.65	2.28	1	7
Stickleback	1.90	2.10	1	7
Stoat	2.00	2.08	1	7
Sturgeon	2.00	2.04	1	7
Swan	4.27	1.82	1	7
Swift ~	1.70	1.89	1	7
Sword fish ~	2.71	2.09	1	7
Tapir	1.26	1.62	1	7
Tarantula ~ *	2.74	2.29	1	7
Tench	1.69	1.90	1	7
Thrush ~	2.49	2.07	1	7
Tiger	4.39	1.92	1	7
Toad	3.36	2.06	1	7
Tortoise	3.52	2.04	1	7
Trout	3.26	1.88	1	7
Tuna	4.27	1.69	1	7
Turkey	4.38	1.84	1	7
Turtle ~	3.92	1.94	1	7
Tyrannosaurus ~	2.55	2.43	1	7
Vole	2.13	2.01	1	7
Vulture	2.81	2.03	1	7
Wallaby	1.78	2.24	1	7
Walrus	2.44	2.32	1	7
Warthog	2.50	1.99	1	7
Wasp	3.48	2.19	1	7
Water buffalo #	2.09	1.97	1	7
Water rat #	1.93	1.84	1	7
Weasel	2.71	2.08	1	7
Whale ~	3.99	2.01	1	7
White tiger	2.40	2.41	1	7
Wild boar	2.28	2.10	1	7
Wild cat	2.37	2.17	1	7
Wild dog	2.22	2.23	1	7
Wildebeest	2.36	2.02	1	7
Wolf ~	3.74	2.01	1	7
Wombat	2.12	2.18	1	7
Woodcock	1.84	1.90	1	7

Animal	Mean	SD	Range min	max
Woodlouse	3.53	1.92	1	7
Woodpecker	2.49	2.20	1	7
Worm	3.99	1.99	1	7
Wren ~	1.82	1.93	1	7
Yak	1.81	1.80	1	7
Yellow tit	1.89	1.88	1	7
Yellowhammer ~	1.33	1.73	1	7
Yorkshire terrier	4.91	1.41	2	7
Zebra	3.45	2.15	1	7
Zebu	1.35	1.63	1	7

Table B.2b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to the 21-30 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	2.33	2.26	1	7
Almond	3.49	2.01	1	7
Apple	5.60	1.27	1	7
Apricot	4.12	1.79	1	7
Aubergine	3.15	2.13	1	7
Avocado	3.44	2.02	1	7
Banana	5.37	1.43	1	7
Berries	5.20	1.52	2	7
Bilberry #	1.45	1.75	1	7
Blackberry	5.03	1.54	2	7
Blackcurrant	4.58	1.68	1	7
Blueberry	3.29	2.13	1	7
Bramble #	2.80	2.07	1	7
Butternut squash	3.20	2.15	1	7
Cantaloupe melon	2.81	2.16	1	7
Cherry	4.63	1.62	1	7
Chestnut	3.63	1.79	1	7
Citron	1.89	2.23	1	7
Clementine # ~	4.90	1.48	2	7
Coconut	4.59	1.70	1	7
Cox apple #	4.19	1.75	1	7
Crab apple	3.12	2.06	1	7
Cranberry	4.26	1.68	1	7
Cucumber	4.13	1.95	1	7
Currant	4.27	1.69	1	7
Damson #	1.50	1.93	1	6
Date #	3.06	2.18	1	7
Dewberry	1.56	1.91	1	7
Durian	1.17	1.54	1	7
Elderberry #	2.26	2.03	1	7
Fig	2.71	2.21	1	7
Gala apple	3.46	2.18	1	7
Galia melon ~	2.54	2.10	1	7
Gooseberry	2.54	2.31	1	7
Granny smith	4.17	2.00	1	7
Grapefruit	4.10	1.95	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	Range max
Grapes	5.85	1.29	2	7
Green melon	2.32	2.21	1	7
Guava	1.96	2.18	1	7
Haw #	1.17	1.54	1	7
Hazelnut	4.08	1.80	1	7
Honeydew melon ~	3.08	2.28	1	7
Horse chestnut	2.83	2.17	1	7
Jackfruit	1.31	1.47	1	7
Jaffa #	2.09	2.21	1	7
Kiwi ~	4.37	1.77	1	7
Kumquat	1.64	1.71	1	7
Lemon	5.67	1.34	3	7
Lime #	4.35	1.75	1	7
Loganberry #	1.60	1.91	1	7
Lychee	2.04	2.36	1	7
Mandarin ~	4.28	1.67	1	7
Mango #	4.08	1.99	1	7
Medlar	1.28	1.66	1	7
Melon	4.47	1.84	1	7
Nectarine ~	3.83	1.93	1	7
Nuts	4.54	1.70	1	7
Olive	4.37	1.90	1	7
Orange	5.19	1.55	1	7
Papaya	2.18	2.24	1	7
Passion fruit	3.87	1.67	1	7
Paw paw	1.81	1.96	1	7
Peach #	5.30	1.46	2	7
Pear	5.46	1.47	2	7
Pepper	5.96	1.12	3	7
Persimmon	1.37	2.17	1	7
Pineapple	5.05	1.55	1	7
Plantain	1.50	2.10	1	7
Plum	4.44	1.81	1	7
Pomelo	1.14	1.60	1	6
Pomegranate	3.39	1.93	1	7
Prune #	3.25	2.07	1	7
Quince #	1.36	1.53	1	7
Raisin	5.07	1.67	2	7
Rambotan	1.09	0.95	1	4

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	max
Raspberry	4.78	1.65	1	7
Red currant	2.93	1.87	1	7
Redberry #	3.13	1.95	1	7
Red grape	4.48	1.81	1	7
Rhubarb	3.49	2.18	1	7
Rosehip #	1.66	1.88	1	7
Sapodilla	1.11	0.42	1	2
Satsuma ~	5.57	1.41	2	7
Sharon fruit	1.70	2.37	1	7
Sloe berry	1.96	2.41	1	7
Squash	2.26	2.22	1	7
Star fruit	1.92	2.34	1	7
Strawberry	5.34	1.39	1	7
Sultana	3.90	1.95	1	7
Tangelo *	1.12	1.43	1	7
Tangerine	5.15	1.57	2	7
Tayberry	1.17	1.76	1	7
Tomato	4.74	1.61	1	7
Walnut #	3.86	1.92	1	7
Water melon	4.07	1.97	1	7
Whinberry	1.33	1.73	1	7
White currant	1.55	2.06	1	7
White grape	3.25	2.21	1	7
Whortleberry	1.30	1.66	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Table B.3a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to the 31-40 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark	2.16	1.87	1	7
Adder	2.91	1.98	1	7
Albatross	2.05	2.20	1	7
Alligator ~	3.38	1.98	1	7
Alpaca	1.42	1.67	1	7
Amoeba	2.22	1.99	1	7
Angel fish	2.29	1.97	1	7
Ant	4.45	1.93	2	7
Ant bear	1.31	1.54	1	6
Anteater	2.25	2.13	1	7
Antelope	2.61	2.13	1	7
Ape	3.84	1.93	1	7
Armadillo	2.03	2.00	1	7
Ass	2.23	1.88	1	7
Baboon ~	3.31	2.09	1	7
Badger	3.91	1.80	1	7
Bald eagle #	2.18	1.77	1	7
Bat	3.10	2.20	1	7
Bear	3.66	2.05	1	7
Beaver	2.45	2.43	1	7
Bee	3.98	2.01	1	7
Beetle	4.11	1.87	2	7
Bird	6.83	0.37	6	7
Bison #	1.88	2.11	1	6
Blackbird ~	3.70	2.04	1	7
Blue bird	2.05	2.11	1	7
Blue tit ~	3.01	1.93	1	7
Boa constrictor	2.29	1.80	1	6
Boar	2.22	1.85	1	7
Brontosaurus #	2.55	2.19	1	7
Brown bear	3.05	2.21	1	7
Buck # ~ *	2.00	1.90	1	6
Budgerigar ~	3.45	1.68	1	7
Buffalo	2.37	2.21	1	7
Bull	3.63	2.02	1	7
Bullfinch ~	1.80	2.01	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Bullock ~	2.31	2.17	1	7
Butterfly	5.23	1.44	2	7
Buzzard # *	1.97	1.88	1	6
Calf	4.61	1.62	1	7
Camel	2.90	2.26	1	7
Canary	2.72	1.83	1	7
Caribou	1.60	1.58	1	5
Carp	2.12	2.06	1	7
Cat	6.81	0.49	5	7
Caterpillar	3.87	1.97	1	7
Cattle	4.11	1.92	1	7
Chaffinch ~	2.10	2.16	1	7
Chameleon ~	2.63	2.33	1	7
Cheetah ~	3.06	2.19	1	7
Chicken	6.69	0.70	4	7
Chimpanzee	3.62	2.11	1	7
Chinchilla	1.77	1.92	1	7
Chipmunk	2.58	1.97	1	7
Civet	1.07	0.34	1	2
Clown fish ~	2.27	2.20	1	7
Cobra	2.26	2.06	1	7
Cockatiel	2.25	1.83	1	7
Cocker spaniel	3.33	1.88	1	7
Cockerel	3.27	2.17	1	7
Cockroach	2.41	2.25	1	7
Cod	4.57	1.91	2	7
Condor	1.75	1.71	1	7
Conger eel	2.22	2.21	1	7
Cougar #	1.79	1.94	1	6
Cow	6.12	1.10	3	7
Coyote ~ *	2.13	1.92	1	7
Cray fish	1.88	2.23	1	7
Crocodile	3.66	2.06	1	7
Crow	4.00	1.88	1	7
Cuckoo	2.29	2.06	1	7
Curlew ~	1.12	0.43	1	2
Deer	4.55	1.85	2	7
Dingo	1.80	1.81	1	6
Dog	6.83	0.37	6	7

Animal	Mean	SD	Range min	Range max
Dolphin ~	4.16	1.90	1	7
Donkey	5.39	1.54	2	7
Dormouse	2.10	2.05	1	7
Dove	3.44	2.15	1	7
Dragon ~	2.21	2.44	1	7
Dragonfly	2.69	2.29	1	7
Dromedary ~	1.48	2.17	1	7
Duck	5.37	1.49	2	7
Duckbill platypus	1.49	1.47	1	5
Dugong	1.07	0.90	1	4
Duiker	1.07	1.39	1	6
Eagle	3.33	1.94	1	7
Earthworm	3.46	2.06	1	7
Earwig	2.18	1.92	1	7
Echidna	1.09	0.39	1	2
Eel	2.86	2.11	1	7
Elephant	5.11	1.59	2	7
Elk ~	1.95	1.42	1	5
Emu	2.40	1.93	1	7
Ewe ~	4.29	1.83	2	7
Ferret	3.81	1.85	2	7
Field mouse	3.12	1.98	1	7
Finch ~	2.30	2.01	1	7
Fish ~	6.20	0.99	4	7
Flamingo	3.62	1.82	2	7
Flea	2.77	2.20	1	7
Fly	5.30	1.64	2	7
Fowl	2.36	2.13	1	7
Fox	5.01	1.68	2	7
Frog	5.09	1.54	3	7
Gazelle	2.57	2.05	1	7
Gecko	2.32	2.01	1	7
Gerbil	3.55	1.86	1	7
Giant panda	2.59	2.15	1	7
Gibbon	2.50	1.79	1	7
Giraffe	3.80	2.12	1	7
Gnu	1.53	1.42	1	5
Goat	5.08	1.67	2	7
Goldcrest	1.51	1.86	1	7

Animal	Mean	SD	Range min	Range max
Goldfish	4.58	1.85	2	7
Goose	3.21	2.20	1	7
Gorilla	5.11	1.56	2	7
Greyhound	3.27	2.05	1	7
Grizzly bear	3.15	1.99	1	7
Groundhog #	1.48	1.37	1	5
Guinea fowl	1.99	1.92	1	7
Guinea pig	3.47	1.99	1	7
Gull ~	4.59	1.57	2	7
Haddock	4.36	1.80	2	7
Hamster ~	3.64	1.85	1	7
Hare	4.17	1.81	2	7
Hart	1.19	1.49	1	5
Hawk	2.74	1.82	1	7
Hedgehog	3.98	1.87	1	7
Heifer #	2.16	2.11	1	7
Hen	4.85	1.64	2	7
Heron	2.71	2.01	1	7
Herring	2.39	1.90	1	7
Hippopotamus	3.28	2.17	1	7
Hornet	2.04	1.92	1	6
Horse	4.96	1.59	1	7
Horsefly	1.92	1.50	1	6
Hyena	2.58	2.10	1	7
Ibex	1.22	1.53	1	6
Iguana	2.40	1.85	1	7
Impala	1.98	1.85	1	6
Insect	5.94	1.19	3	7
Invertebrate # ~ *	2.66	2.08	1	7
Jack rabbit # *	1.72	1.58	1	6
Jackal	2.12	1.98	1	7
Jackass	1.57	1.82	1	6
Jackdaw ~	1.78	1.97	1	7
Jaguar	2.76	2.04	1	7
Jerboa	1.08	0.56	1	3
Kangaroo	3.49	2.13	1	7
Kid	2.74	1.84	1	7
Kitten	4.56	1.80	1	7
Kiwi	1.60	1.86	1	7

Animal	Mean	SD	Range min	max
Koala	3.29	2.00	1	7
Koi carp	3.17	1.76	1	7
Komodo dragon # *	2.24	2.12	1	7
Lacewing	1.26	0.73	1	3
Lady bird	4.74	1.60	2	7
Lamb	5.71	1.24	2	7
Lemur	1.99	1.63	1	7
Leopard	3.24	1.98	1	7
Lion	4.07	1.85	1	7
Lizard	3.36	2.02	1	7
Llama	2.81	2.05	1	7
Lobster	2.24	2.47	1	7
Long tailed tit	1.30	1.94	1	7
Lynx	2.10	1.67	1	6
Mackerel	3.10	2.04	1	7
Mammal ~	4.72	1.72	1	7
Manatee	1.74	2.08	1	7
Mandrill ~ *	1.12	1.00	1	5
Mangust	1.00	0.00	1	1
Marmoset *	1.48	0.75	1	3
Marmot	1.18	1.10	1	5
Marten	1.60	1.77	1	7
Meerkat	2.31	2.30	1	7
Midge	2.23	2.20	1	7
Mink *	1.86	1.83	1	6
Minnow # *	1.40	1.32	1	5
Manx	1.62	1.66	1	6
Mole	2.94	1.98	1	7
Mongoose #	1.62	1.80	1	7
Monkey ~	4.65	1.69	1	7
Moose ~	2.31	1.95	1	7
Moth	4.18	1.73	1	7
Mouse	4.35	1.70	1	7
Mule	2.92	1.98	1	7
Musk ox ~ *	1.11	1.12	1	5
Newt	2.14	2.22	1	7
Nightingale	1.96	2.01	1	7
Ocelot	1.23	1.12	1	5
Octopus ~	3.30	2.25	1	7

~ significant effect of age, **#** significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Orang-utan	3.80	1.86	1	7
Oryx	1.24	0.96	1	4
Ostrich	3.30	1.77	1	7
Otter	3.11	2.08	1	7
Owl	3.82	2.13	1	7
Ox	2.42	2.09	1	7
Panda	3.02	2.36	1	7
Panther	2.77	1.96	1	7
Parakeet	2.12	1.81	1	7
Parrot	3.90	2.05	2	7
Partridge	2.60	1.88	1	7
Peacock	4.16	1.78	2	7
Peewit ~	1.04	0.27	1	2
Pelican	2.80	1.97	1	7
Penguin	3.69	2.00	1	7
Perch	1.73	1.95	1	7
Pheasant	3.07	2.11	1	7
Pig	6.19	1.05	3	7
Pigeon	5.92	1.02	4	7
Piglet	3.99	1.79	1	7
Pike #	2.19	1.98	1	7
Pine marten	1.52	1.93	1	7
Piranha fish #	2.42	2.25	1	7
Plaice	3.65	1.61	1	7
Platypus	1.82	2.12	1	7
Polar bear	3.74	2.14	1	7
Polar cat	1.40	0.70	1	3
Pony	5.45	1.41	2	7
Porcupine	1.97	2.00	1	7
Porpoise *	2.07	1.86	1	7
Poultry	5.35	1.43	2	7
Prairie dog	1.67	1.50	1	5
Puffin	2.02	2.28	1	7
Puma	2.15	2.20	1	7
Rabbit	6.33	0.83	5	7
Racoon *	2.28	1.70	1	7
Ram	2.96	1.86	1	7
Rat	4.17	1.96	1	7
Ratel	1.00	0.00	1	1

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Raven	2.91	1.97	1	7
Reindeer	3.52	2.18	1	7
Rhesus monkey	1.84	1.90	1	7
Rhinoceros	4.58	1.77	2	7
Roach	1.72	2.09	1	7
Robin	4.40	1.74	2	7
Rodent	3.29	2.03	1	7
Roe deer ~	1.84	1.91	1	7
Rook # ~	2.72	2.01	1	7
Rooster	3.66	1.81	1	7
Salamander	2.17	1.47	1	7
Salmon	4.16	1.90	1	7
Sardine	2.73	2.02	1	7
Sea lion	2.67	2.13	1	7
Seagull	4.35	1.87	1	7
Seahorse	2.64	2.19	1	7
Seal	3.16	2.02	1	7
Shark ~	3.54	2.05	1	7
Sheep	5.29	1.55	2	7
Short tailed tit	1.44	1.74	1	7
Shrew	1.87	1.75	1	7
Shrimp	2.89	2.19	1	7
Siamese cat	2.89	1.93	1	7
Siberian tiger ~	2.05	2.21	1	7
Skate	2.02	1.87	1	7
Skunk	2.30	2.23	1	7
Skylark ~	1.72	1.90	1	7
Sloth # ~	1.94	1.68	1	6
Slug	3.53	2.19	1	7
Snail	3.42	2.05	1	7
Snake ~	3.81	2.03	1	7
Sole ~	1.87	1.74	1	6
Sow ~	2.44	2.15	1	7
Sparrow ~	4.54	1.63	2	7
Spider	6.42	0.69	5	7
Springbok	2.12	1.97	1	7
Squid	2.46	2.13	1	7
Squirrel	6.14	0.92	4	7
Stag	2.55	1.90	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Star fish	2.85	2.37	1	7
Starling ~	2.38	2.42	1	7
Stick insect	2.49	2.06	1	7
Stickleback	1.92	1.89	1	7
Stoat	1.81	1.80	1	6
Sturgeon	1.99	1.96	1	7
Swan	4.01	2.04	1	7
Swift ~	1.81	1.92	1	7
Sword fish ~	2.72	1.79	1	7
Tapir	1.54	1.77	1	7
Tarantula ~ *	3.32	2.06	1	7
Tench	1.57	1.79	1	7
Thrush ~	2.85	1.93	1	7
Tiger	3.82	2.11	1	7
Toad	2.75	2.27	1	7
Tortoise	3.72	1.84	1	7
Trout	2.59	2.03	1	7
Tuna	5.48	1.24	3	7
Turkey	5.25	1.52	3	7
Turtle ~	3.39	1.90	1	7
Tyrannosaurus ~	2.83	2.32	1	7
Vole	2.20	1.63	1	6
Vulture	2.23	2.42	1	7
Wallaby	2.50	1.84	1	7
Walrus	3.32	1.78	1	7
Warthog	2.42	1.87	1	7
Wasp	4.05	1.98	1	7
Water buffalo #	1.84	1.92	1	7
Water rat #	1.70	1.84	1	7
Weasel	2.17	2.04	1	6
Whale ~	4.10	1.80	1	7
White tiger	1.95	1.70	1	6
Wild boar	1.99	1.84	1	6
Wild cat	1.87	1.91	1	7
Wild dog	1.99	1.86	1	6
Wildebeest	2.24	2.18	1	7
Wolf ~	3.89	1.91	1	7
Wombat	2.18	1.59	1	7
Woodcock	1.73	1.76	1	6

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Woodlouse	2.51	2.13	1	7
Woodpecker	2.73	1.86	1	7
Worm	4.75	1.81	2	7
Wren ~	2.26	1.89	1	6
Yak	1.74	1.75	1	5
Yellow tit	1.56	1.80	1	7
Yellowhammer ~	1.36	1.81	1	7
Yorkshire terrier	4.43	1.77	2	7
Zebra	4.01	2.01	2	7
Zebu	1.05	0.53	1	3

Table B.3b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to the 31-40 age –category.

Fruit	**Mean**	**SD**	**Range** min	**Range** max
Acorn	2.64	2.24	1	7
Almond	3.23	2.28	1	7
Apple	6.86	0.45	5	7
Apricot	4.98	1.50	2	7
Aubergine	3.44	1.89	1	7
Avocado	3.11	2.32	1	7
Banana	6.56	0.89	3	7
Berries	5.11	1.69	2	7
Bilberry #	1.20	1.12	1	4
Blackberry	4.27	1.87	1	7
Blackcurrant	4.37	1.73	1	7
Blueberry	3.27	2.23	1	7
Bramble #	3.53	2.02	1	7
Butternut squash	4.18	1.93	2	7
Cantaloupe melon	3.40	2.24	1	7
Cherry	5.57	1.44	2	7
Chestnut	3.26	2.15	1	7
Citron	1.69	2.02	1	7
Clementine # ~	5.61	1.38	3	7
Coconut	4.71	1.70	1	7
Cox apple #	4.27	1.73	1	7
Crab apple	2.17	2.43	1	7
Cranberry	4.37	1.89	2	7
Cucumber	4.67	1.71	1	7
Currant	4.94	1.64	2	7
Damson #	1.86	2.22	1	7
Date #	2.93	2.11	1	7
Dewberry	1.22	1.82	1	7
Durian	1.16	0.87	1	4
Elderberry #	1.94	1.90	1	7
Fig	3.37	1.87	1	7
Gala apple	3.27	2.34	1	7
Galia melon ~	3.03	2.35	1	7
Gooseberry	3.48	2.13	1	7
Granny smith	5.22	1.42	2	7
Grapefruit	4.92	1.68	2	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	Range max
Grapes	6.72	0.44	6	7
Green melon	2.29	2.55	1	7
Guava	1.93	2.32	1	7
Haw #	1.31	1.53	1	5
Hazelnut	3.51	2.10	1	7
Honeydew melon ~	3.67	2.17	1	7
Horse chestnut	3.18	1.93	1	7
Jackfruit	1.25	1.98	1	6
Jaffa #	3.16	2.21	1	7
Kiwi ~	4.14	1.68	1	7
Kumquat	1.69	1.68	1	7
Lemon	6.09	1.14	3	7
Lime #	4.50	1.81	1	7
Loganberry #	1.51	1.60	1	5
Lychee	2.36	2.10	1	7
Mandarin ~	4.26	1.92	1	7
Mango #	3.66	2.05	1	7
Medlar	1.14	0.61	1	3
Melon	4.71	1.76	1	7
Nectarine ~	6.05	1.02	4	7
Nuts	4.81	1.81	2	7
Olive	4.36	1.94	1	7
Orange	6.89	0.31	6	7
Papaya	2.59	2.05	1	7
Passion fruit	3.38	1.84	1	7
Paw paw	1.71	2.21	1	7
Peach #	6.32	0.83	4	7
Pear	6.32	0.95	4	7
Pepper	5.84	1.34	2	7
Persimmon	1.42	2.10	1	7
Pineapple	6.40	0.89	4	7
Plantain	1.44	2.44	1	7
Plum	5.79	1.21	3	7
Pomelo	1.18	1.20	1	5
Pomegranate	3.43	1.99	1	7
Prune #	3.22	2.04	1	7
Quince #	1.64	1.59	1	5
Raisin	5.42	1.55	2	7
Rambotan	1.07	1.15	1	5

Fruit	Mean	SD	Range min	Range max
Raspberry	4.66	1.68	1	7
Red currant	2.85	1.95	1	7
Redberry #	2.02	2.18	1	7
Red grape	6.47	0.70	5	7
Rhubarb	4.48	1.85	2	7
Rosehip #	1.87	1.85	1	7
Sapodilla	1.13	1.51	1	5
Satsuma ~	5.84	1.21	3	7
Sharon fruit	1.50	2.10	1	7
Sloe berry	1.45	1.56	1	6
Squash	2.59	2.35	1	7
Star fruit	2.43	2.00	1	7
Strawberry	6.50	0.75	5	7
Sultana	4.95	1.62	2	7
Tangelo *	1.14	1.51	1	6
Tangerine	6.32	0.95	4	7
Tayberry	1.07	1.39	1	6
Tomato	6.59	0.73	4	7
Walnut #	3.17	2.24	1	7
Water melon	4.07	1.93	1	7
Whinberry	1.04	0.29	1	2
White currant	1.29	0.92	1	4
White grape	4.07	2.06	1	7
Whortleberry	1.08	0.36	1	2

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table B.4a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to the 41-50 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark	2.88	2.38	1	7
Adder	3.40	2.05	1	7
Albatross	2.53	2.46	1	7
Alligator ~	4.19	2.08	1	7
Alpaca	2.97	2.06	1	7
Amoeba	3.25	2.36	1	7
Angel fish	1.98	2.61	1	7
Ant	4.22	2.21	2	7
Ant bear	1.34	2.27	1	7
Anteater	2.49	2.56	1	7
Antelope	3.72	2.15	1	7
Ape	4.02	2.14	1	7
Armadillo	2.48	2.52	1	7
Ass	2.59	2.47	1	7
Baboon ~	3.64	2.31	1	7
Badger	3.99	2.06	1	7
Bald eagle #	2.51	2.40	1	7
Bat	4.11	2.04	1	7
Bear	3.53	2.30	1	7
Beaver	3.84	2.21	1	7
Bee	5.69	1.48	3	7
Beetle	3.31	2.51	1	7
Bird	5.97	1.28	3	7
Bison #	2.36	2.48	1	7
Blackbird ~	5.58	1.44	3	7
Blue bird	2.49	2.59	1	7
Blue tit ~	4.54	1.91	2	7
Boa constrictor	3.04	2.36	1	7
Boar	2.98	2.53	1	7
Brontosaurus #	3.15	2.31	1	7
Brown bear	3.15	2.41	1	7
Buck # ~ *	2.45	2.44	1	7
Budgerigar ~	3.63	2.16	1	7
Buffalo	3.94	1.93	1	7
Bull	5.16	1.75	2	7
Bullfinch ~	2.46	2.49	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Bullock ~	3.85	2.02	1	7
Butterfly	4.06	1.98	1	7
Buzzard # *	3.07	2.16	1	7
Calf	4.10	1.95	1	7
Camel	3.81	2.21	1	7
Canary	3.73	2.12	1	7
Caribou	2.19	2.38	1	7
Carp	2.30	2.27	1	7
Cat	7.00	0.00	7	7
Caterpillar	3.76	2.24	1	7
Cattle	3.90	1.96	1	7
Chaffinch ~	3.21	2.24	1	7
Chameleon ~	2.55	2.56	1	7
Cheetah ~	4.16	1.97	2	7
Chicken	4.82	1.60	1	7
Chimpanzee	3.96	2.17	1	7
Chinchilla	2.62	2.53	1	7
Chipmunk	2.94	2.31	1	7
Civet	1.15	1.60	1	7
Clown fish ~	1.48	2.31	1	7
Cobra	2.95	2.31	1	7
Cockatiel	3.10	2.25	1	7
Cocker spaniel	3.75	2.12	1	7
Cockerel	3.23	2.22	1	7
Cockroach	3.84	2.10	1	7
Cod	4.84	1.83	3	7
Condor	2.33	2.13	1	7
Conger eel	2.22	2.57	1	7
Cougar #	2.80	2.41	1	7
Cow	5.88	1.34	2	7
Coyote ~ *	2.01	2.66	1	7
Cray fish	2.45	2.44	1	7
Crocodile	4.56	2.02	2	7
Crow	4.93	1.65	3	7
Cuckoo	3.76	2.03	1	7
Curlew ~	1.80	2.44	1	7
Deer	4.47	1.86	1	7
Dingo	2.42	2.32	1	7
Dog	6.64	0.80	4	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Dolphin ~	5.39	1.63	2	7
Donkey	4.60	1.75	1	7
Dormouse	2.60	2.59	1	7
Dove	4.84	1.86	2	7
Dragon ~	3.10	2.44	1	7
Dragonfly	3.84	1.98	1	7
Dromedary ~	2.00	2.38	1	7
Duck	6.65	0.61	5	7
Duckbill platypus	2.25	2.45	1	7
Dugong	1.15	0.63	1	3
Duiker	1.21	1.85	1	7
Eagle	3.81	2.21	1	7
Earthworm	3.39	2.29	1	7
Earwig	2.92	2.38	1	7
Echidna	1.21	2.24	1	7
Eel	3.90	1.98	1	7
Elephant	4.37	1.90	1	7
Elk ~	2.29	2.46	1	7
Emu	3.68	2.16	1	7
Ewe ~	3.44	2.23	1	7
Ferret	3.07	2.26	1	7
Field mouse	3.31	2.31	1	7
Finch ~	3.13	2.26	1	7
Fish ~	6.32	1.08	3	7
Flamingo	3.09	2.42	1	7
Flea	4.67	1.87	2	7
Fly	5.91	1.27	3	7
Fowl	2.90	2.37	1	7
Fox	4.26	1.97	1	7
Frog	4.35	1.89	1	7
Gazelle	4.19	1.67	2	7
Gecko	2.31	2.06	1	7
Gerbil	2.72	2.54	1	7
Giant panda	4.08	2.12	1	7
Gibbon	3.31	2.22	1	7
Giraffe	4.91	1.85	2	7
Gnu	1.99	2.49	1	7
Goat	3.96	2.17	1	7
Goldcrest	1.48	1.84	1	7

Animal	Mean	SD	Range min	max
Goldfish	4.11	2.04	1	7
Goose	4.11	2.06	1	7
Gorilla	3.40	2.52	1	7
Greyhound	3.72	2.24	1	7
Grizzly bear	3.33	2.33	1	7
Groundhog #	2.27	2.14	1	7
Guinea fowl	2.31	2.10	1	7
Guinea pig	3.05	2.42	1	7
Gull ~	4.60	1.75	1	7
Haddock	3.55	2.16	1	7
Hamster ~	3.90	2.14	1	7
Hare	3.84	2.17	1	7
Hart	1.21	1.16	1	5
Hawk	4.05	2.03	1	7
Hedgehog	5.28	1.64	2	7
Heifer #	2.80	2.36	1	7
Hen	4.39	1.86	1	7
Heron	3.25	2.18	1	7
Herring	3.65	2.13	1	7
Hippopotamus	4.22	1.86	1	7
Hornet	3.09	2.22	1	7
Horse	5.82	1.34	2	7
Horsefly	3.18	2.10	1	7
Hyena	3.23	2.25	1	7
Ibex	1.96	2.09	1	7
Iguana	2.39	2.43	1	7
Impala	1.93	2.44	1	7
Insect	6.11	1.19	4	7
Invertebrate # ~ *	2.42	2.31	1	7
Jack rabbit # *	2.15	2.33	1	7
Jackal	3.36	2.16	1	7
Jackass	2.57	2.40	1	7
Jackdaw ~	2.19	2.53	1	7
Jaguar	3.45	2.18	1	7
Jerboa	1.38	2.10	1	7
Kangaroo	4.86	1.90	2	7
Kid	3.49	2.22	1	7
Kitten	4.32	2.04	1	7
Kiwi	2.39	2.37	1	7

Animal	Mean	SD	Range min	Range max
Koala	3.76	2.24	1	7
Koi carp	2.41	2.55	1	7
Komodo dragon # *	2.17	2.56	1	7
Lacewing	1.34	2.37	1	7
Lady bird	3.95	2.10	1	7
Lamb	4.29	1.98	1	7
Lemur	1.92	2.47	1	7
Leopard	3.73	2.13	1	7
Lion	4.62	1.70	1	7
Lizard	3.62	2.27	1	7
Llama	2.68	2.44	1	7
Lobster	3.96	1.92	1	7
Long tailed tit	2.05	2.21	1	7
Lynx	2.60	2.20	1	7
Mackerel	3.25	2.27	1	7
Mammal ~	5.03	1.69	2	7
Manatee	1.93	2.20	1	7
Mandrill ~ *	1.61	2.87	1	7
Mangust	1.12	1.66	1	7
Marmoset *	2.19	2.02	1	7
Marmot	1.70	1.59	1	7
Marten	1.56	1.29	1	5
Meerkat	4.03	1.91	1	7
Midge	2.78	2.15	1	7
Mink *	2.47	2.42	1	7
Minnow # *	2.07	2.62	1	7
Manx	2.60	2.60	1	7
Mole	3.81	2.21	1	7
Mongoose #	2.90	2.34	1	7
Monkey ~	4.19	2.06	1	7
Moose ~	3.52	2.23	1	7
Moth	4.19	2.08	1	7
Mouse	4.69	1.98	2	7
Mule	3.65	2.13	1	7
Musk ox ~ *	1.68	2.25	1	7
Newt	3.50	2.14	1	7
Nightingale	2.98	2.10	1	7
Ocelot	1.78	2.23	1	7
Octopus ~	3.33	2.30	1	7

Animal	Mean	SD	Range min	Range max
Orang-utan	3.33	2.21	1	7
Oryx	1.41	2.34	1	7
Ostrich	3.31	2.41	1	7
Otter	4.70	1.95	2	7
Owl	3.39	2.27	1	7
Ox	3.81	2.03	1	7
Panda	3.61	2.22	1	7
Panther	2.98	2.50	1	7
Parakeet	2.44	2.58	1	7
Parrot	3.83	2.24	1	7
Partridge	3.75	1.99	1	7
Peacock	3.92	2.17	1	7
Peewit ~	1.22	0.78	1	3
Pelican	3.40	2.37	1	7
Penguin	4.16	2.24	2	7
Perch	1.83	2.58	1	7
Pheasant	4.19	1.96	1	7
Pig	4.69	1.63	1	7
Pigeon	4.78	1.96	2	7
Piglet	3.76	2.24	1	7
Pike #	2.99	2.27	1	7
Pine marten	1.97	1.92	1	7
Piranha fish #	2.66	2.55	1	7
Plaice	3.65	1.98	1	7
Platypus	2.69	2.43	1	7
Polar bear	3.89	2.27	1	7
Polar cat	1.51	2.26	1	7
Pony	4.35	1.88	1	7
Porcupine	2.79	2.48	1	7
Porpoise *	3.59	2.26	1	7
Poultry	4.11	2.04	1	7
Prairie dog	2.21	2.41	1	7
Puffin	3.88	2.21	2	7
Puma	2.85	2.38	1	7
Rabbit	4.91	1.85	2	7
Racoon *	3.03	2.27	1	7
Ram	3.85	2.02	1	7
Rat	4.25	2.03	1	7
Ratel	1.26	1.89	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Raven	3.21	2.21	1	7
Reindeer	5.16	1.75	2	7
Rhesus monkey	2.25	2.30	1	7
Rhinoceros	3.55	2.31	1	7
Roach	3.05	2.16	1	7
Robin	5.37	1.67	2	7
Rodent	4.02	2.13	1	7
Roe deer ~	1.59	2.67	1	7
Rook # ~	3.38	2.28	1	7
Rooster	3.84	2.05	1	7
Salamander	2.67	2.27	1	7
Salmon	5.03	1.67	2	7
Sardine	3.15	2.15	1	7
Sea lion	3.16	2.34	1	7
Seagull	5.66	1.45	2	7
Seahorse	3.02	2.43	1	7
Seal	4.16	2.03	1	7
Shark ~	4.22	2.01	1	7
Sheep	4.86	1.61	1	7
Short tailed tit	1.97	2.04	1	7
Shrew	2.68	2.27	1	7
Shrimp	3.23	2.28	1	7
Siamese cat	3.41	2.21	1	7
Siberian tiger ~	2.24	2.48	1	7
Skate	2.79	2.40	1	7
Skunk	2.70	2.55	1	7
Skylark ~	2.55	2.21	1	7
Sloth # ~	3.00	2.17	1	7
Slug	4.71	1.94	2	7
Snail	5.28	1.64	2	7
Snake ~	4.34	2.10	2	7
Sole ~	2.45	2.41	1	7
Sow ~	3.91	1.88	1	7
Sparrow ~	5.40	1.54	3	7
Spider	6.26	1.09	4	7
Springbok	2.76	2.37	1	7
Squid	3.09	2.45	1	7
Squirrel	6.03	1.28	3	7
Stag	4.07	1.95	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Star fish	3.49	2.22	1	7
Starling ~	4.73	1.86	2	7
Stick insect	2.85	2.53	1	7
Stickleback	2.29	2.73	1	7
Stoat	3.00	2.30	1	7
Sturgeon	2.20	2.17	1	7
Swan	3.84	2.21	1	7
Swift ~	3.03	2.27	1	7
Sword fish ~	2.98	2.36	1	7
Tapir	1.84	2.11	1	7
Tarantula ~ *	3.09	2.37	1	7
Tench	2.05	2.47	1	7
Thrush ~	3.70	1.98	1	7
Tiger	5.52	1.52	3	7
Toad	4.97	1.81	2	7
Tortoise	3.87	2.24	1	7
Trout	4.29	2.02	1	7
Tuna	5.39	1.63	2	7
Turkey	5.50	1.53	2	7
Turtle ~	3.78	2.28	1	7
Tyrannosaurus ~	2.77	2.46	1	7
Vole	2.69	2.43	1	7
Vulture	3.73	2.20	1	7
Wallaby	3.02	2.43	1	7
Walrus	3.62	2.22	1	7
Warthog	2.44	2.40	1	7
Wasp	5.89	1.33	3	7
Water buffalo #	3.38	2.19	1	7
Water rat #	2.50	2.30	1	7
Weasel	3.45	2.11	1	7
Whale ~	4.02	2.13	1	7
White tiger	2.48	1.91	1	7
Wild boar	3.62	1.85	1	7
Wild cat	2.47	2.40	1	7
Wild dog	3.32	2.06	1	7
Wildebeest	2.95	2.31	1	7
Wolf ~	3.20	2.42	1	7
Wombat	2.91	2.13	1	7
Woodcock	2.25	2.14	1	7

Animal	Mean	SD	Range min	Range max
Woodlouse	2.42	2.45	1	7
Woodpecker	4.13	2.09	2	7
Worm	5.71	1.41	3	7
Wren ~	3.21	2.10	1	7
Yak	2.15	2.49	1	7
Yellow tit	3.18	1.83	1	7
Yellowhammer ~	1.38	1.73	1	7
Yorkshire terrier	4.16	2.05	1	7
Zebra	5.28	1.72	2	7
Zebu	1.21	0.94	1	4

Table B.4b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to the 41-50 age –category.

Fruit	**Mean**	**SD**	**Range** min	max
Acorn	3.58	2.18	1	7
Almond	4.65	1.72	1	7
Apple	7.00	0.00	7	7
Apricot	4.38	1.79	1	7
Aubergine	3.93	2.22	2	7
Avocado	3.44	2.42	1	7
Banana	7.00	0.00	7	7
Berries	4.58	1.98	2	7
Bilberry #	1.77	2.73	1	7
Blackberry	4.71	1.68	1	7
Blackcurrant	5.95	1.20	3	7
Blueberry	3.68	2.31	1	7
Bramble #	2.65	2.35	1	7
Butternut squash	2.79	2.56	1	7
Cantaloupe melon	3.27	2.15	1	7
Cherry	5.68	1.50	2	7
Chestnut	4.15	1.79	1	7
Citron	2.05	2.35	1	7
Clementine # ~	3.70	2.21	1	7
Coconut	4.36	1.94	1	7
Cox apple #	4.53	1.95	2	7
Crab apple	3.73	2.04	1	7
Cranberry	6.36	0.78	5	7
Cucumber	5.45	1.51	2	7
Currant	4.64	1.66	1	7
Damson #	2.30	2.29	1	7
Date #	3.73	2.21	1	7
Dewberry	1.85	2.24	1	7
Durian	1.14	1.80	1	7
Elderberry #	3.53	2.01	1	7
Fig	3.21	2.29	1	7
Gala apple	3.94	1.93	1	7
Galia melon ~	2.29	2.64	1	7
Gooseberry	5.40	1.54	3	7
Granny smith	5.80	1.29	4	7
Grapefruit	4.29	1.99	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	max
Grapes	6.40	0.94	4	7
Green melon	3.01	2.09	1	7
Guava	2.06	2.31	1	7
Haw #	1.31	1.70	1	7
Hazelnut	5.26	1.69	2	7
Honeydew melon ~	4.20	1.96	1	7
Horse chestnut	3.38	2.03	1	7
Jackfruit	1.35	1.85	1	7
Jaffa #	4.71	1.79	2	7
Kiwi ~	3.81	2.05	1	7
Kumquat	2.26	2.43	1	7
Lemon	6.32	1.08	3	7
Lime #	5.05	1.83	2	7
Loganberry #	2.18	2.41	1	7
Lychee	2.98	2.40	1	7
Mandarin ~	3.39	2.25	1	7
Mango #	4.09	2.03	1	7
Medlar	1.18	1.70	1	7
Melon	4.43	1.88	1	7
Nectarine ~	4.26	2.21	2	7
Nuts	6.24	1.02	4	7
Olive	3.92	2.17	1	7
Orange	6.73	0.58	5	7
Papaya	2.56	2.33	1	7
Passion fruit	3.50	2.18	1	7
Paw paw	2.13	2.18	1	7
Peach #	4.74	1.93	2	7
Pear	4.72	1.74	1	7
Pepper	4.21	2.02	1	7
Persimmon	1.59	2.25	1	7
Pineapple	6.13	1.09	3	7
Plantain	1.97	2.14	1	7
Plum	4.34	1.88	1	7
Pomelo	1.21	1.85	1	7
Pomegranate	2.72	2.63	1	7
Prune #	4.06	1.98	1	7
Quince #	1.99	2.15	1	7
Raisin	5.56	1.42	2	7
Rambotan	1.22	1.65	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range	
			min	max
Raspberry	5.63	1.46	3	7
Red currant	2.72	2.44	1	7
Redberry #	2.47	2.20	1	7
Red grape	4.23	1.94	1	7
Rhubarb	4.32	1.85	1	7
Rosehip #	1.82	2.37	1	7
Sapodilla	1.09	0.71	1	3
Satsuma ~	5.19	1.56	2	7
Sharon fruit	1.82	2.22	1	7
Sloe berry	1.69	2.40	1	7
Squash	3.97	2.02	2	7
Star fruit	2.20	2.33	1	7
Strawberry	6.73	0.58	5	7
Sultana	4.48	1.73	1	7
Tangelo *	1.22	1.64	1	7
Tangerine	4.92	1.81	2	7
Tayberry	1.13	1.00	1	4
Tomato	7.00	0.00	7	7
Walnut #	5.76	1.42	3	7
Water melon	4.53	1.99	2	7
Whinberry	1.16	0.87	1	4
White currant	1.80	2.09	1	7
White grape	3.03	2.27	1	7
Whortleberry	1.09	0.39	1	2

Table B.5a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to the 51-60 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	1.53	1.90	1	7
Adder	2.80	2.15	1	7
Albatross	2.26	2.27	1	7
Alligator ~	2.72	2.39	1	7
Alpaca	2.00	2.25	1	7
Amoeba	1.81	1.96	1	7
Angel fish	1.96	2.09	1	7
Ant	4.18	1.97	1	7
Ant bear	1.19	0.92	1	4
Anteater	2.09	2.06	1	7
Antelope	2.24	2.46	1	7
Ape	3.46	2.13	1	7
Armadillo	2.04	2.32	1	7
Ass	3.19	1.89	1	7
Baboon ~	2.69	2.14	1	7
Badger	4.17	1.87	2	7
Bald eagle #	2.06	2.29	1	7
Bat	3.54	1.93	1	7
Bear	3.87	2.19	2	7
Beaver	3.17	2.08	1	7
Bee	4.91	1.68	2	7
Beetle	3.76	2.14	1	7
Bird	4.65	1.75	1	7
Bison #	2.18	2.38	1	7
Blackbird ~	3.80	1.91	1	7
Blue bird	2.04	2.39	1	7
Blue tit ~	3.11	2.22	1	7
Boa constrictor	2.57	2.18	1	7
Boar	2.37	2.50	1	7
Brontosaurus #	1.44	1.95	1	7
Brown bear	3.25	2.21	1	7
Buck # ~ *	1.89	2.24	1	7
Budgerigar ~	2.63	2.32	1	7
Buffalo	2.65	2.34	1	7
Bull	3.58	2.12	1	7
Bullfinch ~	2.24	2.36	1	7

Animal	Mean	SD	Range min	max
Bullock ~	3.33	1.91	1	7
Butterfly	4.18	2.12	2	7
Buzzard # *	1.86	2.20	1	7
Calf	4.87	1.69	2	7
Camel	3.59	2.06	1	7
Canary	2.86	2.29	1	7
Caribou	1.64	2.32	1	7
Carp	2.86	2.07	1	7
Cat	4.98	1.50	1	7
Caterpillar	3.46	2.07	1	7
Cattle	5.25	1.57	2	7
Chaffinch ~	2.53	2.31	1	7
Chameleon ~	2.15	2.12	1	7
Cheetah ~	3.30	2.31	1	7
Chicken	4.89	1.61	1	7
Chimpanzee	3.44	2.26	1	7
Chinchilla	2.59	2.37	1	7
Chipmunk	2.16	2.06	1	6
Civet	1.27	1.38	1	6
Clown fish ~	1.49	2.17	1	7
Cobra	2.20	2.46	1	7
Cockatiel	3.63	1.82	1	7
Cocker spaniel	4.39	1.77	1	7
Cockerel	4.56	1.65	2	7
Cockroach	2.72	2.44	1	7
Cod	4.39	1.78	1	7
Condor	1.88	2.33	1	7
Conger eel	1.96	2.05	1	7
Cougar #	1.87	2.42	1	7
Cow	4.75	1.70	1	7
Coyote ~ *	1.78	2.35	1	7
Cray fish	1.99	2.27	1	7
Crocodile	3.49	2.14	1	7
Crow	3.41	2.32	1	7
Cuckoo	2.62	2.22	1	7
Curlew ~	1.86	1.98	1	7
Deer	4.77	1.70	2	7
Dingo	2.34	1.87	1	7
Dog	4.98	1.58	1	7

Animal	Mean	SD	Range min	Range max
Dolphin ~	4.24	1.85	2	7
Donkey	4.24	1.99	2	7
Dormouse	3.42	1.83	1	7
Dove	3.43	2.16	1	7
Dragon ~	2.16	2.50	1	7
Dragonfly	3.04	1.93	1	7
Dromedary ~	1.88	2.24	1	7
Duck	4.23	1.93	1	7
Duckbill platypus	1.95	1.93	1	7
Dugong	1.15	1.09	1	5
Duiker	1.00	0.00	1	1
Eagle	3.59	2.14	1	7
Earthworm	4.36	1.90	2	7
Earwig	2.76	2.47	1	7
Echidna	1.26	2.00	1	7
Eel	2.83	2.14	1	7
Elephant	3.14	2.32	1	7
Elk ~	2.44	2.32	1	7
Emu	2.53	2.15	1	7
Ewe ~	3.20	2.27	1	7
Ferret	4.27	1.73	2	7
Field mouse	3.72	1.91	1	7
Finch ~	3.05	2.12	1	7
Fish ~	3.80	2.11	1	7
Flamingo	3.14	2.05	1	7
Flea	2.56	2.55	1	7
Fly	4.11	2.08	1	7
Fowl	3.26	2.07	1	7
Fox	5.34	1.39	3	7
Frog	3.42	2.06	1	7
Gazelle	2.55	2.19	1	7
Gecko	1.86	2.09	1	6
Gerbil	3.44	1.69	1	7
Giant panda	3.57	2.09	1	7
Gibbon	2.13	2.12	1	7
Giraffe	3.18	2.33	1	7
Gnu	1.69	2.05	1	7
Goat	4.12	1.99	2	7
Goldcrest	1.53	2.06	1	7

Animal	Mean	SD	Range min	Range max
Goldfish	4.33	2.10	2	7
Goose	3.66	2.03	1	7
Gorilla	3.36	2.35	1	7
Greyhound	4.72	1.70	2	7
Grizzly bear	3.00	2.25	1	7
Groundhog #	1.38	1.71	1	7
Guinea fowl	2.38	1.94	1	7
Guinea pig	3.72	1.90	1	7
Gull ~	4.28	2.03	2	7
Haddock	4.39	1.78	1	7
Hamster ~	3.61	1.85	1	7
Hare	3.95	1.77	2	7
Hart	1.57	2.27	1	7
Hawk	3.35	2.00	1	7
Hedgehog	4.18	1.94	1	7
Heifer #	2.73	2.26	1	7
Hen	4.27	1.90	1	7
Heron	3.01	2.12	1	7
Herring	3.34	1.79	1	7
Hippopotamus	3.40	2.24	2	7
Hornet	3.17	1.87	1	7
Horse	4.79	1.67	1	7
Horsefly	2.24	2.00	1	7
Hyena	2.67	1.98	1	7
Ibex	1.57	2.32	1	7
Iguana	2.19	2.12	1	7
Impala	1.45	1.96	1	6
Insect	3.47	2.37	1	7
Invertebrate # ~ *	1.90	2.63	1	7
Jack rabbit # *	1.82	2.50	1	7
Jackal	2.00	2.09	1	7
Jackass	1.78	2.36	1	7
Jackdaw ~	2.63	2.34	1	7
Jaguar	2.85	2.43	1	7
Jerboa	1.29	2.15	1	7
Kangaroo	3.01	2.32	1	7
Kid	3.26	2.18	1	7
Kitten	5.68	1.30	2	7
Kiwi	1.93	2.20	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Koala	2.98	2.15	1	7
Koi carp	2.69	2.26	1	7
Komodo dragon # *	1.71	2.52	1	7
Lacewing	1.49	1.96	1	7
Lady bird	3.79	1.96	1	7
Lamb	5.34	1.50	2	7
Lemur	1.79	1.58	1	5
Leopard	3.47	2.32	1	7
Lion	4.20	1.92	2	7
Lizard	3.29	2.15	1	7
Llama	2.67	2.25	1	7
Lobster	3.97	1.84	1	7
Long tailed tit	1.81	2.31	1	7
Lynx	1.75	2.51	1	7
Mackerel	2.92	2.14	1	7
Mammal ~	3.51	2.21	1	7
Manatee	1.33	2.04	1	7
Mandrill ~ *	1.27	1.59	1	5
Mangust	1.11	0.75	1	3
Marmoset *	1.55	1.70	1	6
Marmot	1.35	1.85	1	7
Marten	1.65	1.82	1	6
Meerkat	3.44	2.11	1	7
Midge	2.75	2.09	1	7
Mink *	2.41	2.41	1	7
Minnow # *	2.16	2.25	1	7
Manx	1.88	2.42	1	7
Mole	4.02	1.79	1	7
Mongoose #	2.29	2.24	1	7
Monkey ~	3.84	2.11	1	7
Moose ~	2.16	2.71	1	7
Moth	3.21	2.26	1	7
Mouse	4.37	1.59	1	7
Mule	2.76	2.12	1	7
Musk ox ~ *	1.32	2.00	1	7
Newt	2.79	1.89	1	7
Nightingale	2.53	2.06	1	7
Ocelot	1.44	2.00	1	7
Octopus ~	3.12	2.20	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Orang-utan	2.87	2.39	1	7
Oryx	1.37	1.71	1	7
Ostrich	3.09	2.05	1	7
Otter	2.92	2.20	1	7
Owl	3.71	1.94	1	7
Ox	2.42	2.37	1	7
Panda	2.93	2.37	1	7
Panther	2.89	2.35	1	7
Parakeet	2.49	1.93	1	7
Parrot	3.57	2.09	1	7
Partridge	3.37	1.87	1	7
Peacock	4.06	1.90	2	7
Peewit ~	1.74	2.10	1	7
Pelican	2.17	2.33	1	7
Penguin	4.27	1.94	2	7
Perch	1.92	2.08	1	7
Pheasant	4.86	1.70	2	7
Pig	4.73	1.65	1	7
Pigeon	4.21	1.96	1	7
Piglet	4.17	1.70	1	7
Pike #	2.22	1.97	1	7
Pine marten	1.65	1.85	1	6
Piranha fish #	2.52	2.19	1	7
Plaice	4.23	1.85	2	7
Platypus	1.67	1.92	1	6
Polar bear	3.11	2.31	1	7
Polar cat	1.28	1.90	1	6
Pony	5.06	1.57	2	7
Porcupine	2.08	2.47	1	7
Porpoise *	2.04	2.27	1	7
Poultry	5.17	1.59	2	7
Prairie dog	2.04	2.09	1	7
Puffin	2.13	2.37	1	7
Puma	2.79	2.18	1	7
Rabbit	4.54	1.87	1	7
Racoon *	2.17	2.36	1	7
Ram	3.12	2.15	1	7
Rat	3.37	2.14	1	7
Ratel	1.07	1.15	1	5

Animal	Mean	SD	Range min	Range max
Raven	2.80	2.15	1	7
Reindeer	4.37	1.81	2	7
Rhesus monkey	2.63	1.84	1	7
Rhinoceros	2.75	2.23	1	7
Roach	1.58	2.22	1	7
Robin	4.28	1.98	1	7
Rodent	3.84	2.07	1	7
Roe deer ~	3.02	2.08	1	7
Rook # ~	2.57	2.48	1	7
Rooster	3.47	2.12	1	7
Salamander	1.75	1.95	1	7
Salmon	3.84	2.24	1	7
Sardine	2.42	2.24	1	7
Sea lion	3.04	2.10	1	7
Seagull	4.28	1.90	1	7
Seahorse	2.44	2.38	1	7
Seal	4.45	1.87	2	7
Shark ~	3.49	2.08	1	7
Sheep	4.51	1.74	1	7
Short tailed tit	1.77	1.82	1	7
Shrew	2.12	2.18	1	7
Shrimp	4.38	1.87	2	7
Siamese cat	4.77	1.38	3	7
Siberian tiger ~	2.26	2.24	1	7
Skate	2.48	2.13	1	7
Skunk	2.19	2.39	1	7
Skylark ~	2.46	2.14	1	7
Sloth # ~	2.09	2.22	1	7
Slug	3.57	2.08	1	7
Snail	3.70	2.17	1	7
Snake ~	3.97	1.96	1	7
Sole ~	3.02	2.04	1	7
Sow ~	2.85	2.24	1	7
Sparrow ~	4.68	2.00	2	7
Spider	4.48	1.89	1	7
Springbok	1.65	2.14	1	7
Squid	3.67	1.95	1	7
Squirrel	4.45	1.81	1	7
Stag	3.06	2.12	1	7

Animal	Mean	SD	Range min	Range max
Star fish	2.47	2.08	1	7
Starling ~	4.26	1.97	1	7
Stick insect	2.18	2.24	1	7
Stickleback	2.41	2.23	1	7
Stoat	2.12	2.32	1	7
Sturgeon	1.85	2.09	1	7
Swan	3.65	2.06	1	7
Swift ~	3.01	2.32	1	7
Sword fish ~	2.49	2.02	1	7
Tapir	1.88	1.91	1	7
Tarantula ~ *	1.92	2.30	1	7
Tench	1.58	2.19	1	7
Thrush ~	3.79	2.21	1	7
Tiger	3.85	2.20	2	7
Toad	3.80	1.73	1	7
Tortoise	3.45	2.17	1	7
Trout	4.20	1.62	2	7
Tuna	3.18	2.22	1	7
Turkey	5.00	1.58	2	7
Turtle ~	3.00	2.29	1	7
Tyrannosaurus ~	1.33	2.31	1	7
Vole	2.48	2.20	1	7
Vulture	2.53	2.30	1	7
Wallaby	2.75	2.23	1	7
Walrus	3.03	2.15	1	7
Warthog	1.71	2.44	1	7
Wasp	4.28	1.89	1	7
Water buffalo #	1.81	2.28	1	7
Water rat #	2.57	1.98	1	7
Weasel	2.18	2.21	1	7
Whale ~	3.30	2.16	1	7
White tiger	2.00	2.59	1	7
Wild boar	2.75	2.27	1	7
Wild cat	2.18	2.66	1	7
Wild dog	2.41	2.09	1	7
Wildebeest	2.35	2.14	1	7
Wolf ~	3.05	2.18	1	7
Wombat	2.12	2.46	1	7
Woodcock	1.97	2.42	1	7

Animal	**Mean**	**SD**	**Range**	
			min	**max**
Woodlouse	3.56	2.08	1	7
Woodpecker	3.59	1.98	1	7
Worm	3.39	2.24	1	7
Wren ~	3.32	2.03	1	7
Yak	1.73	2.16	1	7
Yellow tit	1.76	2.25	1	7
Yellowhammer ~	1.70	2.28	1	7
Yorkshire terrier	4.15	1.87	1	7
Zebra	3.49	2.22	1	7
Zebu	1.40	2.17	1	7

Table B.5b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to the 51-60 age –category.

Fruit	**Mean**	**SD**	**Range**	
			min	**max**
Acorn	2.27	2.73	1	7
Almond	4.62	1.62	1	7
Apple	6.22	1.12	3	7
Apricot	5.05	1.58	3	7
Aubergine	3.58	2.06	1	7
Avocado	4.81	1.66	2	7
Banana	6.56	0.70	5	7
Berries	5.42	1.54	2	7
Bilberry #	2.36	1.79	1	7
Blackberry	4.72	1.84	2	7
Blackcurrant	5.04	1.58	2	7
Blueberry	3.53	2.02	1	7
Bramble #	4.14	1.97	1	7
Butternut squash	4.37	1.72	1	7
Cantaloupe melon	2.70	2.34	1	7
Cherry	5.55	1.36	2	7
Chestnut	3.60	2.14	1	7
Citron	2.06	2.43	1	7
Clementine # ~	5.64	1.20	3	7
Coconut	5.30	1.45	2	7
Cox apple #	5.70	1.42	3	7
Crab apple	2.82	2.28	1	7
Cranberry	4.95	1.58	2	7
Cucumber	4.51	1.81	1	7
Currant	4.62	1.62	1	7
Damson #	3.35	2.06	1	7
Date #	3.70	1.97	1	7
Dewberry	1.51	1.69	1	6
Durian	1.24	0.80	1	3
Elderberry #	3.17	2.06	1	7
Fig	3.92	1.91	1	7
Gala apple	3.40	2.03	1	7
Galia melon ~	3.94	1.96	1	7
Gooseberry	3.55	2.11	1	7
Granny smith	5.70	1.14	4	7
Grapefruit	4.76	1.94	2	7

Fruit	Mean	SD	Range min	Range max
Grapes	6.44	0.87	4	7
Green melon	1.88	2.75	1	7
Guava	2.00	1.55	1	5
Haw #	1.46	2.47	1	7
Hazelnut	5.08	1.57	2	7
Honeydew melon ~	4.38	1.93	1	7
Horse chestnut	2.73	2.45	1	7
Jackfruit	1.10	1.08	1	5
Jaffa #	5.15	1.59	2	7
Kiwi ~	5.44	1.25	3	7
Kumquat	2.13	2.32	1	7
Lemon	5.20	1.58	2	7
Lime #	3.27	2.05	1	7
Loganberry #	2.11	2.33	1	7
Lychee	2.73	2.19	1	7
Mandarin ~	5.18	1.51	2	7
Mango #	4.16	2.01	2	7
Medlar	1.16	0.87	1	4
Melon	5.79	1.35	3	7
Nectarine ~	5.33	1.60	2	7
Nuts	5.83	1.28	2	7
Olive	4.48	1.71	1	7
Orange	6.22	1.12	3	7
Papaya	2.48	1.92	1	6
Passion fruit	2.91	2.04	1	7
Paw paw	2.29	1.85	1	7
Peach #	4.90	1.71	2	7
Pear	5.56	1.53	2	7
Pepper	4.66	1.75	1	7
Persimmon	1.37	1.51	1	5
Pineapple	5.68	1.36	3	7
Plantain	2.02	2.17	1	7
Plum	4.62	1.64	1	7
Pomelo	1.25	1.68	1	6
Pomegranate	4.35	1.99	2	7
Prune #	4.30	1.74	1	7
Quince #	1.82	2.05	1	7
Raisin	5.66	1.35	2	7
Rambotan	1.12	0.89	1	4

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	Range max
Raspberry	5.99	1.16	3	7
Red currant	4.46	1.64	2	7
Redberry #	2.06	2.09	1	7
Red grape	4.10	2.03	1	7
Rhubarb	5.61	1.37	3	7
Rosehip #	2.48	2.15	1	7
Sapodilla	1.15	1.63	1	5
Satsuma ~	5.72	1.31	2	7
Sharon fruit	1.68	1.86	1	7
Sloe berry	2.61	1.86	1	6
Squash	3.28	2.43	1	7
Star fruit	2.47	2.30	1	7
Strawberry	6.44	0.87	4	7
Sultana	4.60	1.59	1	7
Tangelo *	1.16	0.94	1	4
Tangerine	5.35	1.44	2	7
Tayberry	1.58	1.78	1	7
Tomato	6.00	1.23	2	7
Walnut #	4.44	1.72	1	7
Water melon	5.51	1.39	3	7
Whinberry	1.16	0.77	1	3
White currant	1.90	1.79	1	7
White grape	3.36	2.23	1	7
Whortleberry	1.16	0.77	1	3

Table B.6a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to the 61-70 age –category.

Animal	**Mean**	**SD**	**Range** min	**Range** max
Aardvark	1.67	2.27	1	7
Adder	1.76	2.39	1	7
Albatross	2.18	2.14	1	7
Alligator ~	2.37	2.46	1	7
Alpaca	2.01	1.99	1	7
Amoeba	1.76	2.37	1	7
Angel fish	1.84	2.06	1	7
Ant	2.93	2.52	1	7
Ant bear	1.40	1.57	1	7
Anteater	1.76	2.20	1	7
Antelope	2.25	2.36	1	7
Ape	2.63	2.48	1	7
Armadillo	2.10	2.27	1	7
Ass	2.53	2.17	1	7
Baboon ~	2.04	2.62	1	7
Badger	3.09	2.24	1	7
Bald eagle #	1.82	2.47	1	7
Bat	2.34	2.40	1	7
Bear	3.01	2.37	1	7
Beaver	2.31	2.39	1	7
Bee	3.50	2.20	1	7
Beetle	2.29	2.43	1	7
Bird	3.83	2.14	1	7
Bison #	2.39	2.27	1	7
Blackbird ~	3.37	2.26	1	7
Blue bird	2.09	2.24	1	7
Blue tit ~	2.43	2.37	1	7
Boa constrictor	1.89	2.05	1	7
Boar	1.87	2.48	1	7
Brontosaurus #	1.61	2.33	1	7
Brown bear	2.81	2.36	1	7
Buck # ~ *	1.63	2.77	1	7
Budgerigar ~	3.35	2.25	1	7
Buffalo	2.27	2.63	1	7
Bull	3.43	2.19	1	7
Bullfinch ~	2.03	2.06	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Bullock ~	3.33	2.21	1	7
Butterfly	3.72	2.29	1	7
Buzzard # *	1.99	2.50	1	7
Calf	3.19	2.54	1	7
Camel	2.82	2.32	1	7
Canary	2.92	2.24	1	7
Caribou	1.75	2.11	1	7
Carp	2.07	2.16	1	7
Cat	4.91	1.63	1	7
Caterpillar	2.65	2.48	1	7
Cattle	4.62	1.82	1	7
Chaffinch ~	2.35	2.25	1	7
Chameleon ~	1.80	2.12	1	7
Cheetah ~	1.99	2.47	1	7
Chicken	5.39	1.56	2	7
Chimpanzee	2.75	2.52	1	7
Chinchilla	2.31	2.17	1	7
Chipmunk	1.72	2.34	1	7
Civet	1.38	2.05	1	7
Clown fish ~	1.50	2.00	1	7
Cobra	1.85	2.24	1	7
Cockatiel	2.16	2.31	1	7
Cocker spaniel	4.31	1.94	1	7
Cockerel	2.53	2.45	1	7
Cockroach	2.02	2.26	1	7
Cod	3.49	2.14	1	7
Condor	1.49	2.06	1	7
Conger eel	1.80	2.20	1	7
Cougar #	2.02	2.32	1	7
Cow	4.41	1.94	1	7
Coyote ~ *	1.65	2.21	1	7
Cray fish	1.63	2.16	1	7
Crocodile	2.57	2.33	1	7
Crow	2.83	2.27	1	7
Cuckoo	2.70	2.20	1	7
Curlew ~	1.93	2.37	1	7
Deer	4.01	2.04	1	7
Dingo	1.85	2.35	1	7
Dog	5.47	1.57	2	7

Animal	Mean	SD	Range min	max
Dolphin ~	2.20	2.47	1	7
Donkey	3.89	2.08	1	7
Dormouse	2.41	2.46	1	7
Dove	3.41	2.27	1	7
Dragon ~	1.88	2.33	1	7
Dragonfly	2.43	2.45	1	7
Dromedary ~	1.94	2.34	1	7
Duck	4.08	1.89	1	7
Duckbill platypus	1.56	1.77	1	6
Dugong	1.17	1.66	1	7
Duiker	1.35	2.20	1	7
Eagle	2.11	2.46	1	7
Earthworm	2.67	2.54	1	7
Earwig	2.53	2.52	1	7
Echidna	1.30	1.81	1	7
Eel	2.11	2.41	1	7
Elephant	3.27	2.38	1	7
Elk ~	2.11	2.29	1	7
Emu	2.02	2.48	1	7
Ewe ~	3.52	2.21	1	7
Ferret	2.20	2.14	1	7
Field mouse	2.72	2.41	1	7
Finch ~	2.57	2.15	1	7
Fish ~	3.80	2.14	1	7
Flamingo	2.29	2.49	1	7
Flea	2.34	2.60	1	7
Fly	3.68	2.12	1	7
Fowl	3.15	2.12	1	7
Fox	3.05	2.38	1	7
Frog	2.89	2.49	1	7
Gazelle	2.04	2.54	1	7
Gecko	1.56	2.09	1	7
Gerbil	2.10	2.27	1	7
Giant panda	2.78	2.38	1	7
Gibbon	1.97	2.48	1	7
Giraffe	3.51	2.24	1	7
Gnu	1.56	2.17	1	7
Goat	3.31	2.26	1	7
Goldcrest	1.77	1.85	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Goldfish	3.65	2.27	1	7
Goose	2.91	2.24	1	7
Gorilla	2.60	2.61	1	7
Greyhound	3.45	2.20	1	7
Grizzly bear	2.72	2.27	1	7
Groundhog #	1.35	1.68	1	7
Guinea fowl	1.65	2.01	1	7
Guinea pig	2.98	2.19	1	7
Gull ~	2.92	2.42	1	7
Haddock	3.30	2.25	1	7
Hamster ~	3.38	2.17	1	7
Hare	3.44	2.01	1	7
Hart	1.60	2.09	1	7
Hawk	2.57	2.25	1	7
Hedgehog	3.43	2.27	1	7
Heifer #	2.76	2.31	1	7
Hen	4.03	2.04	1	7
Heron	2.55	2.24	1	7
Herring	2.15	2.26	1	7
Hippopotamus	2.60	2.36	1	7
Hornet	2.23	2.17	1	7
Horse	4.33	1.91	1	7
Horsefly	1.90	2.21	1	7
Hyena	2.02	2.32	1	7
Ibex	1.73	1.92	1	7
Iguana	1.82	2.23	1	7
Impala	1.98	2.30	1	7
Insect	3.21	2.32	1	7
Invertebrate # ~ *	1.79	2.40	1	7
Jack rabbit # *	1.82	2.54	1	7
Jackal	1.89	2.26	1	7
Jackass	1.59	2.21	1	7
Jackdaw ~	2.20	1.94	1	7
Jaguar	2.32	2.37	1	7
Jerboa	1.13	1.71	1	7
Kangaroo	2.57	2.48	1	7
Kid	2.51	2.29	1	7
Kitten	4.19	2.02	1	7
Kiwi	1.73	2.41	1	7

Animal	Mean	SD	Range min	Range max
Koala	2.12	2.55	1	7
Koi carp	2.15	2.17	1	7
Komodo dragon # *	1.59	2.44	1	7
Lacewing	1.34	1.75	1	7
Lady bird	3.66	2.12	1	7
Lamb	5.34	1.51	2	7
Lemur	1.84	2.45	1	7
Leopard	2.54	2.29	1	7
Lion	3.06	2.38	1	7
Lizard	2.30	2.19	1	7
Llama	2.19	2.44	1	7
Lobster	2.33	2.39	1	7
Long tailed tit	1.97	2.04	1	7
Lynx	1.81	2.46	1	7
Mackerel	2.68	2.12	1	7
Mammal ~	3.25	2.24	1	7
Manatee	1.66	2.05	1	7
Mandrill ~ *	1.51	2.32	1	7
Mangust	1.04	0.29	1	2
Marmoset *	1.79	2.19	1	7
Marmot	1.59	2.17	1	7
Marten	1.71	2.07	1	7
Meerkat	2.03	2.74	1	7
Midge	2.26	2.65	1	7
Mink *	1.81	2.22	1	7
Minnow # *	1.68	2.17	1	7
Manx	1.49	1.91	1	7
Mole	2.22	2.39	1	7
Mongoose #	2.26	2.40	1	7
Monkey ~	3.21	2.22	1	7
Moose ~	2.08	2.36	1	7
Moth	2.61	2.55	1	7
Mouse	3.47	2.00	1	7
Mule	2.43	2.30	1	7
Musk ox ~ *	1.74	2.29	1	7
Newt	1.81	2.17	1	7
Nightingale	2.42	1.90	1	7
Ocelot	1.64	2.41	1	7
Octopus ~	2.25	2.35	1	7

Animal	Mean	SD	Range min	Range max
Orang-utan	2.68	2.33	1	7
Oryx	1.33	2.19	1	7
Ostrich	2.37	2.48	1	7
Otter	2.18	2.56	1	7
Owl	2.87	2.39	1	7
Ox	2.42	2.31	1	7
Panda	2.34	2.39	1	7
Panther	2.36	2.55	1	7
Parakeet	1.97	2.20	1	7
Parrot	2.60	2.31	1	7
Partridge	2.49	2.08	1	7
Peacock	3.65	2.12	1	7
Peewit ~	1.74	2.08	1	7
Pelican	1.90	2.39	1	7
Penguin	2.51	2.57	1	7
Perch	2.11	2.11	1	7
Pheasant	3.25	2.03	1	7
Pig	3.62	2.17	1	7
Pigeon	3.75	2.14	1	7
Piglet	3.22	2.38	1	7
Pike #	2.14	2.25	1	7
Pine marten	1.54	2.11	1	7
Piranha fish #	1.58	1.92	1	7
Plaice	3.36	2.23	1	7
Platypus	1.67	2.47	1	7
Polar bear	2.96	2.40	1	7
Polar cat	1.42	1.81	1	7
Pony	4.44	1.95	2	7
Porcupine	1.83	2.45	1	7
Porpoise *	2.04	2.10	1	7
Poultry	4.16	1.95	1	7
Prairie dog	1.87	2.04	1	7
Puffin	2.33	2.58	1	7
Puma	1.96	2.31	1	7
Rabbit	4.85	1.86	2	7
Racoon *	1.77	2.54	1	7
Ram	2.25	2.55	1	7
Rat	2.65	2.29	1	7
Ratel	1.04	0.29	1	2

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Raven	2.26	2.38	1	7
Reindeer	3.29	2.18	1	7
Rhesus monkey	2.57	2.39	1	7
Rhinoceros	2.76	2.34	1	7
Roach	1.52	2.34	1	7
Robin	4.03	1.98	1	7
Rodent	3.41	2.04	1	7
Roe deer ~	2.57	2.03	1	7
Rook # ~	2.69	2.36	1	7
Rooster	3.06	2.14	1	7
Salamander	1.70	1.95	1	7
Salmon	3.73	2.13	1	7
Sardine	2.92	2.33	1	7
Sea lion	2.58	2.30	1	7
Seagull	3.26	2.40	1	7
Seahorse	1.90	2.27	1	7
Seal	3.33	2.24	1	7
Shark ~	2.27	2.47	1	7
Sheep	3.70	2.19	1	7
Short tailed tit	1.48	1.09	1	4
Shrew	1.75	1.90	1	7
Shrimp	2.68	2.25	1	7
Siamese cat	2.55	2.22	1	7
Siberian tiger ~	1.99	2.28	1	7
Skate	1.97	2.26	1	7
Skunk	1.81	2.55	1	7
Skylark ~	2.27	2.17	1	7
Sloth # ~	1.74	2.25	1	7
Slug	2.43	2.37	1	7
Snail	2.93	2.33	1	7
Snake ~	2.05	2.65	1	7
Sole ~	2.06	2.19	1	7
Sow ~	3.55	2.24	1	7
Sparrow ~	3.80	2.06	1	7
Spider	2.48	2.65	1	7
Springbok	2.10	2.14	1	7
Squid	1.83	2.03	1	7
Squirrel	4.10	2.01	1	7
Stag	3.04	2.22	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Star fish	1.81	2.61	1	7
Starling ~	3.30	2.12	1	7
Stick insect	2.02	2.03	1	7
Stickleback	1.59	2.49	1	7
Stoat	1.81	2.31	1	7
Sturgeon	1.62	1.85	1	7
Swan	3.50	2.08	1	7
Swift ~	2.79	2.27	1	7
Sword fish ~	1.76	2.42	1	7
Tapir	1.83	2.24	1	7
Tarantula ~ *	1.77	2.41	1	7
Tench	1.76	1.94	1	7
Thrush ~	3.51	2.12	1	7
Tiger	2.77	2.41	1	7
Toad	2.58	2.43	1	7
Tortoise	2.56	2.45	1	7
Trout	2.50	2.32	1	7
Tuna	2.88	2.31	1	7
Turkey	4.09	1.82	1	7
Turtle ~	2.01	2.60	1	7
Tyrannosaurus ~	1.63	2.50	1	7
Vole	2.13	2.12	1	7
Vulture	1.91	2.24	1	7
Wallaby	2.31	2.54	1	7
Walrus	2.12	2.43	1	7
Warthog	1.93	2.04	1	7
Wasp	2.68	2.55	1	7
Water buffalo #	2.02	2.34	1	7
Water rat #	1.74	2.35	1	7
Weasel	2.37	2.20	1	7
Whale ~	2.71	2.33	1	7
White tiger	2.01	2.40	1	7
Wild boar	2.10	2.31	1	7
Wild cat	1.87	2.36	1	7
Wild dog	2.01	2.27	1	7
Wildebeest	1.84	2.40	1	7
Wolf ~	2.21	2.59	1	7
Wombat	1.74	2.46	1	7
Woodcock	1.57	2.06	1	7

Animal	Mean	SD	Range min	max
Woodlouse	2.17	2.45	1	7
Woodpecker	2.47	2.30	1	7
Worm	2.75	2.50	1	7
Wren ~	2.83	2.23	1	7
Yak	1.57	1.93	1	7
Yellow tit	1.99	1.98	1	7
Yellowhammer ~	1.76	1.99	1	7
Yorkshire terrier	4.02	2.01	1	7
Zebra	3.03	2.44	1	7
Zebu	1.32	2.14	1	7

Table B.6b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to the 61-70 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	1.81	2.41	1	7
Almond	2.73	2.33	1	7
Apple	4.33	1.93	1	7
Apricot	3.35	2.15	1	7
Aubergine	2.24	2.09	1	7
Avocado	2.76	2.38	1	7
Banana	4.85	1.63	1	7
Berries	3.57	2.16	1	7
Bilberry #	1.79	2.26	1	7
Blackberry	4.66	1.76	2	7
Blackcurrant	3.80	2.11	1	7
Blueberry	2.57	2.30	1	7
Bramble #	2.90	2.31	1	7
Butternut squash	2.28	2.15	1	7
Cantaloupe melon	3.03	2.16	1	7
Cherry	3.47	2.24	1	7
Chestnut	3.37	1.95	1	7
Citron	1.45	2.09	1	7
Clementine # ~	2.83	2.43	1	7
Coconut	2.46	2.41	1	7
Cox apple #	4.14	1.97	1	7
Crab apple	1.94	2.30	1	7
Cranberry	2.32	2.46	1	7
Cucumber	4.51	1.82	2	7
Currant	3.11	2.25	1	7
Damson #	2.74	2.20	1	7
Date #	3.28	2.15	1	7
Dewberry	1.23	1.72	1	7
Durian	1.15	1.78	1	7
Elderberry #	2.00	2.28	1	7
Fig	2.61	2.15	1	7
Gala apple	2.62	2.38	1	7
Galia melon ~	2.37	2.38	1	7
Gooseberry	2.64	2.40	1	7
Granny smith	3.99	2.00	1	7
Grapefruit	4.42	1.72	2	7

Fruit	Mean	SD	Range min	Range max
Grapes	4.39	1.93	1	7
Green melon	2.54	2.46	1	7
Guava	1.58	2.00	1	7
Haw #	1.40	2.22	1	7
Hazelnut	4.48	1.78	2	7
Honeydew melon ~	4.75	1.72	2	7
Horse chestnut	2.12	2.20	1	7
Jackfruit	1.17	0.97	1	4
Jaffa #	3.93	2.03	1	7
Kiwi ~	3.03	2.29	1	7
Kumquat	1.65	2.33	1	7
Lemon	4.11	1.92	1	7
Lime #	2.59	2.16	1	7
Loganberry #	2.34	2.06	1	7
Lychee	1.74	2.30	1	7
Mandarin ~	4.57	1.60	1	7
Mango #	2.16	2.51	1	7
Medlar	1.12	1.66	1	7
Melon	4.33	1.76	1	7
Nectarine ~	3.78	2.13	1	7
Nuts	4.33	1.78	1	7
Olive	3.47	1.98	1	7
Orange	5.93	1.33	3	7
Papaya	1.70	1.82	1	7
Passion fruit	2.06	2.02	1	7
Paw paw	1.52	1.97	1	7
Peach #	4.29	1.79	1	7
Pear	4.57	1.78	1	7
Pepper	3.67	2.00	1	7
Persimmon	1.37	2.54	1	7
Pineapple	3.79	2.06	1	7
Plantain	1.43	1.84	1	7
Plum	3.86	2.14	1	7
Pomelo	1.20	1.16	1	4
Pomegranate	3.10	1.91	1	7
Prune #	2.66	2.52	1	7
Quince #	1.48	2.16	1	7
Raisin	2.93	2.40	1	7
Rambotan	1.14	1.80	1	7

Fruit	Mean	SD	Range min	max
Raspberry	5.06	1.70	2	7
Red currant	2.93	1.91	1	7
Redberry #	1.25	0.51	1	2
Red grape	3.47	2.25	1	7
Rhubarb	3.88	2.09	1	7
Rosehip #	1.67	2.12	1	7
Sapodilla	1.11	1.39	1	6
Satsuma ~	4.46	1.98	2	7
Sharon fruit	1.23	1.83	1	7
Sloe berry	1.22	1.50	1	7
Squash	2.00	2.33	1	7
Star fruit	1.43	1.73	1	7
Strawberry	4.66	1.75	1	7
Sultana	3.11	2.36	1	7
Tangelo *	1.25	1.48	1	5
Tangerine	3.92	2.06	1	7
Tayberry	1.35	1.67	1	7
Tomato	4.69	1.66	1	7
Walnut #	3.58	2.07	1	7
Water melon	4.64	1.79	2	7
Whinberry	1.15	1.86	1	7
White currant	1.40	1.41	1	5
White grape	2.77	2.37	1	7
Whortleberry	1.32	1.17	1	4

~ significant effect of age, # significant effect of gender, *significant effect of education

Table B.7a. Mean, standard deviation (SD) and range (min-max) familiarity values for animal words in individuals belonging to > 70 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	1.20	1.60	1	7
Adder	1.67	2.28	1	7
Albatross	1.49	1.86	1	7
Alligator ~	2.12	2.37	1	7
Alpaca	1.18	0.63	1	3
Amoeba	1.47	2.22	1	7
Angel fish	1.72	1.99	1	7
Ant	4.46	1.80	2	7
Ant bear	1.15	1.60	1	7
Anteater	1.40	1.59	1	7
Antelope	1.93	2.50	1	7
Ape	2.71	2.16	1	7
Armadillo	1.32	1.69	1	7
Ass	1.78	2.46	1	7
Baboon ~	1.78	2.23	1	7
Badger	3.28	2.13	2	7
Bald eagle #	1.45	1.95	1	7
Bat	1.94	2.33	1	7
Bear	2.47	2.43	1	7
Beaver	1.79	2.16	1	7
Bee	5.36	1.42	3	7
Beetle	3.70	2.11	1	7
Bird	4.36	1.94	1	7
Bison #	1.67	2.02	1	7
Blackbird ~	5.82	1.32	3	7
Blue bird	1.58	2.37	1	7
Blue tit ~	4.80	1.68	2	7
Boa constrictor	1.62	1.91	1	7
Boar	1.55	1.95	1	7
Brontosaurus #	1.26	1.82	1	7
Brown bear	2.14	2.40	1	7
Buck # ~ *	1.82	2.75	1	7
Budgerigar ~	3.75	2.09	2	7
Buffalo	1.61	2.34	1	7
Bull	3.58	2.19	1	7
Bullfinch ~	2.04	1.82	1	7

Animal	Mean	SD	Range min	Range max
Bullock ~	2.51	2.52	1	7
Butterfly	5.42	1.49	2	7
Buzzard # *	1.82	1.78	1	7
Calf	3.38	2.22	1	7
Camel	2.03	2.76	1	7
Canary	2.97	2.21	1	7
Caribou	1.28	1.27	1	5
Carp	2.21	2.02	1	7
Cat	4.87	1.59	1	7
Caterpillar	4.21	2.00	2	7
Cattle	3.86	2.00	1	7
Chaffinch ~	2.62	2.01	1	7
Chameleon ~	1.52	1.95	1	7
Cheetah ~	1.84	2.08	1	7
Chicken	5.08	1.78	3	7
Chimpanzee	2.85	2.26	1	7
Chinchilla	1.47	2.26	1	7
Chipmunk	1.63	1.98	1	6
Civet	1.24	2.15	1	7
Clown fish ~	1.12	1.66	1	7
Cobra	1.44	2.38	1	7
Cockatiel	1.88	2.02	1	7
Cocker spaniel	3.72	1.92	1	7
Cockerel	3.52	2.20	1	7
Cockroach	1.97	2.40	1	7
Cod	2.85	2.37	1	7
Condor	1.22	1.64	1	7
Conger eel	1.34	1.61	1	7
Cougar #	1.68	2.34	1	7
Cow	5.00	1.63	2	7
Coyote ~ *	1.31	1.73	1	7
Cray fish	1.53	2.07	1	7
Crocodile	2.18	2.39	1	7
Crow	4.46	1.91	2	7
Cuckoo	2.27	2.29	1	7
Curlew ~	2.03	1.86	1	7
Deer	3.36	2.22	1	7
Dingo	1.18	1.85	1	7
Dog	6.65	0.61	5	7

Animal	Mean	SD	Range	
			min	max
Dolphin ~	3.49	2.02	2	7
Donkey	3.27	2.17	1	7
Dormouse	2.08	2.36	1	7
Dove	3.97	1.90	1	7
Dragon ~	1.19	2.16	1	7
Dragonfly	2.97	2.18	1	7
Dromedary ~	1.50	1.91	1	7
Duck	5.22	1.58	3	7
Duckbill platypus	1.30	1.66	1	7
Dugong	1.04	0.28	1	2
Duiker	1.07	1.60	1	7
Eagle	2.27	2.28	1	7
Earthworm	5.24	1.40	3	7
Earwig	3.24	2.17	1	7
Echidna	1.11	2.00	1	7
Eel	2.24	2.72	1	7
Elephant	3.86	2.10	2	7
Elk ~	1.55	2.13	1	7
Emu	1.90	2.20	1	7
Ewe ~	2.67	2.26	1	7
Ferret	2.24	2.45	1	7
Field mouse	4.03	2.05	2	7
Finch ~	2.54	2.02	1	7
Fish ~	3.43	2.26	1	7
Flamingo	2.36	2.06	1	7
Flea	2.28	2.54	1	7
Fly	4.85	1.80	2	7
Fowl	3.88	1.94	2	7
Fox	4.16	2.03	2	7
Frog	3.66	2.13	1	7
Gazelle	1.69	2.25	1	7
Gecko	1.40	1.35	1	6
Gerbil	1.89	2.50	1	7
Giant panda	2.37	2.32	1	7
Gibbon	1.44	2.02	1	7
Giraffe	2.06	2.47	1	7
Gnu	1.38	2.24	1	7
Goat	3.06	2.26	1	7
Goldcrest	1.76	2.31	1	7

Animal	Mean	SD	Range min	max
Goldfish	2.80	2.56	1	7
Goose	2.93	2.29	1	7
Gorilla	2.90	2.13	1	7
Greyhound	3.04	2.19	1	7
Grizzly bear	2.44	2.17	1	7
Groundhog #	1.40	1.68	1	7
Guinea fowl	1.46	1.98	1	7
Guinea pig	3.08	2.25	1	7
Gull ~	4.97	1.59	2	7
Haddock	4.05	1.96	1	7
Hamster ~	2.60	2.09	1	7
Hare	3.00	2.14	1	7
Hart	1.38	1.82	1	7
Hawk	3.17	2.06	2	7
Hedgehog	4.21	2.04	2	7
Heifer #	3.26	1.66	2	7
Hen	3.81	2.09	1	7
Heron	2.57	2.41	1	7
Herring	2.25	2.42	1	7
Hippopotamus	2.50	2.53	1	7
Hornet	1.83	1.82	1	7
Horse	5.77	1.13	4	7
Horsefly	1.69	2.21	1	7
Hyena	1.60	1.83	1	7
Ibex	1.18	1.70	1	7
Iguana	1.75	1.70	1	7
Impala	1.26	1.59	1	7
Insect	4.36	1.83	1	7
Invertebrate # ~ *	1.42	1.75	1	7
Jack rabbit # *	1.39	1.88	1	7
Jackal	1.48	1.84	1	7
Jackass	1.12	1.65	1	7
Jackdaw ~	2.62	1.69	1	7
Jaguar	1.88	2.21	1	7
Jerboa	1.15	1.60	1	7
Kangaroo	2.08	2.61	1	7
Kid	1.93	2.32	1	7
Kitten	5.33	1.44	3	7
Kiwi	1.64	2.41	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Koala	2.40	2.02	1	7
Koi carp	2.11	2.28	1	7
Komodo dragon # *	1.11	0.73	1	3
Lacewing	1.43	1.77	1	7
Lady bird	5.03	1.59	2	7
Lamb	5.34	1.55	3	7
Lemur	1.29	1.82	1	7
Leopard	2.04	2.31	1	7
Lion	3.12	2.19	1	7
Lizard	2.12	2.14	1	7
Llama	1.57	2.36	1	7
Lobster	2.34	2.39	1	7
Long tailed tit	1.78	1.91	1	6
Lynx	1.27	0.51	1	2
Mackerel	2.95	2.04	1	7
Mammal ~	2.10	2.66	1	7
Manatee	1.17	1.66	1	7
Mandrill ~ *	1.12	1.09	1	4
Mangust	1.00	0.00	1	1
Marmoset *	1.24	0.85	1	4
Marmot	1.20	1.60	1	7
Marten	1.46	2.02	1	7
Meerkat	2.16	2.62	1	7
Midge	2.06	2.61	1	7
Mink *	1.79	1.95	1	7
Minnow # *	1.61	2.30	1	7
Manx	1.38	1.82	1	7
Mole	2.23	2.31	1	7
Mongoose #	1.49	1.95	1	7
Monkey ~	3.89	1.88	2	7
Moose ~	1.41	1.54	1	6
Moth	3.16	2.13	1	7
Mouse	3.79	2.00	1	7
Mule	2.32	2.25	1	7
Musk ox ~ *	1.23	1.72	1	7
Newt	2.15	2.43	1	7
Nightingale	1.70	2.03	1	6
Ocelot	1.30	1.96	1	7
Octopus ~	1.79	2.07	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Orang-utan	2.06	1.77	1	7
Oryx	1.19	1.79	1	7
Ostrich	2.28	2.40	1	7
Otter	2.99	2.25	1	7
Owl	3.29	2.23	1	7
Ox	1.92	2.50	1	7
Panda	2.87	2.20	1	7
Panther	1.75	2.38	1	7
Parakeet	1.71	2.40	1	7
Parrot	3.30	2.29	2	7
Partridge	2.50	2.19	1	7
Peacock	2.17	2.09	1	7
Peewit ~	1.87	2.40	1	7
Pelican	1.72	1.91	1	7
Penguin	2.90	2.20	1	7
Perch	1.37	1.05	1	4
Pheasant	3.92	2.00	2	7
Pig	5.00	1.68	3	7
Pigeon	5.23	1.62	2	7
Piglet	2.78	2.35	1	7
Pike #	2.09	1.98	1	7
Pine marten	1.42	1.98	1	7
Piranha fish #	1.73	1.98	1	7
Plaice	2.26	2.43	1	7
Platypus	1.37	1.71	1	7
Polar bear	2.62	2.25	1	7
Polar cat	1.07	1.60	1	7
Pony	4.29	2.02	2	7
Porcupine	1.43	2.38	1	7
Porpoise *	1.82	2.22	1	7
Poultry	4.69	1.85	2	7
Prairie dog	1.42	1.85	1	7
Puffin	2.11	2.28	1	7
Puma	1.66	2.38	1	7
Rabbit	4.64	1.80	2	7
Racoon *	1.42	1.98	1	7
Ram	2.32	1.87	1	7
Rat	3.97	2.09	2	7
Ratel	1.07	1.11	1	5

Animal	Mean	SD	Range min	max
Raven	2.75	2.02	1	7
Reindeer	2.10	2.74	1	7
Rhesus monkey	1.54	2.10	1	7
Rhinoceros	2.36	1.98	1	7
Roach	1.80	2.09	1	7
Robin	5.17	1.67	3	7
Rodent	2.78	2.06	1	7
Roe deer ~	2.13	1.68	1	7
Rook # ~	2.54	2.52	1	7
Rooster	3.10	2.26	1	7
Salamander	1.31	1.84	1	7
Salmon	3.80	1.93	2	7
Sardine	2.59	1.92	1	7
Sea lion	1.72	2.02	1	7
Seagull	5.17	1.67	2	7
Seahorse	2.04	2.09	1	7
Seal	2.54	2.27	1	7
Shark ~	2.75	2.26	1	7
Sheep	5.31	1.49	2	7
Short tailed tit	1.98	1.88	1	7
Shrew	1.80	1.99	1	7
Shrimp	2.38	2.37	1	7
Siamese cat	2.34	2.21	1	7
Siberian tiger ~	1.38	1.33	1	5
Skate	1.72	1.87	1	7
Skunk	1.30	2.34	1	7
Skylark ~	2.37	1.95	1	7
Sloth # ~	1.40	2.09	1	7
Slug	3.85	2.09	1	7
Snail	4.21	2.00	2	7
Snake ~	2.49	2.45	1	7
Sole ~	2.33	2.10	1	7
Sow ~	2.64	2.30	1	7
Sparrow ~	5.53	1.47	2	7
Spider	5.03	1.62	2	7
Springbok	1.62	1.95	1	7
Squid	1.76	1.90	1	7
Squirrel	4.85	1.91	2	7
Stag	2.48	1.99	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Star fish	1.72	2.29	1	7
Starling ~	3.62	2.17	1	7
Stick insect	1.89	2.06	1	7
Stickleback	1.83	2.34	1	7
Stoat	1.60	1.95	1	7
Sturgeon	1.42	2.17	1	7
Swan	3.74	1.96	1	7
Swift ~	2.10	2.28	1	7
Sword fish ~	1.32	1.90	1	7
Tapir	1.17	1.66	1	7
Tarantula ~ *	1.35	1.12	1	4
Tench	1.75	1.51	1	6
Thrush ~	3.99	1.86	1	7
Tiger	2.67	2.38	1	7
Toad	3.30	2.06	1	7
Tortoise	2.30	2.66	1	7
Trout	2.99	2.17	1	7
Tuna	2.18	2.42	1	7
Turkey	4.42	1.99	2	7
Turtle ~	2.01	2.11	1	7
Tyrannosaurus ~	1.32	1.98	1	7
Vole	1.91	2.18	1	7
Vulture	1.75	2.30	1	7
Wallaby	1.80	2.58	1	7
Walrus	1.72	2.29	1	7
Warthog	1.50	1.77	1	7
Wasp	4.93	1.73	3	7
Water buffalo #	1.61	2.18	1	7
Water rat #	1.43	2.47	1	7
Weasel	1.86	2.32	1	7
Whale ~	2.34	2.44	1	7
White tiger	1.39	2.42	1	7
Wild boar	1.49	1.98	1	7
Wild cat	1.58	2.10	1	7
Wild dog	1.34	1.33	1	4
Wildebeest	1.62	1.95	1	7
Wolf ~	1.87	2.25	1	7
Wombat	1.30	2.34	1	7
Woodcock	1.61	1.87	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Woodlouse	2.64	2.31	1	7
Woodpecker	2.55	2.25	1	7
Worm	4.98	1.74	2	7
Wren ~	2.95	1.67	1	7
Yak	1.35	2.20	1	7
Yellow tit	1.40	1.69	1	7
Yellowhammer ~	1.76	2.55	1	7
Yorkshire terrier	4.28	1.97	2	7
Zebra	2.34	2.59	1	7
Zebu	1.04	0.29	1	2

Table B.7b. Mean, standard deviation (SD) and range (min-max) familiarity values for fruit words in individuals belonging to > 70 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	2.53	2.58	1	7
Almond	3.64	2.21	1	7
Apple	6.16	1.16	3	7
Apricot	3.81	2.07	1	7
Aubergine	2.64	2.18	1	7
Avocado	3.30	2.27	1	7
Banana	6.15	1.13	3	7
Berries	3.14	2.18	1	7
Bilberry #	2.04	2.11	1	7
Blackberry	3.90	2.02	1	7
Blackcurrant	3.91	1.92	1	7
Blueberry	2.87	2.11	1	7
Bramble #	3.71	2.05	1	7
Butternut squash	2.14	2.26	1	7
Cantaloupe melon	2.05	2.38	1	7
Cherry	4.63	1.72	2	7
Chestnut	3.36	2.06	2	7
Citron	1.72	2.40	1	7
Clementine # ~	2.97	2.13	1	7
Coconut	3.31	2.39	1	7
Cox apple #	5.83	1.39	3	7
Crab apple	2.66	2.49	1	7
Cranberry	4.21	1.83	2	7
Cucumber	4.94	1.79	2	7
Currant	4.80	1.79	3	7
Damson #	3.02	2.35	1	7
Date #	3.30	2.25	1	7
Dewberry	1.49	1.98	1	7
Durian	1.20	1.91	1	7
Elderberry #	3.03	2.17	1	7
Fig	2.81	2.32	1	7
Gala apple	4.18	1.76	1	7
Galia melon ~	2.09	2.20	1	7
Gooseberry	3.87	2.06	1	7
Granny smith	5.79	1.22	4	7
Grapefruit	4.08	1.99	1	7

Fruit	Mean	SD	Range min	Range max
Grapes	4.04	2.13	1	7
Green melon	2.70	2.32	1	7
Guava	1.45	1.73	1	6
Haw #	1.61	1.70	1	6
Hazelnut	3.62	2.12	1	7
Honeydew melon ~	3.44	1.99	1	7
Horse chestnut	2.65	2.34	1	7
Jackfruit	1.06	0.83	1	4
Jaffa #	5.07	1.66	2	7
Kiwi ~	2.84	2.14	1	7
Kumquat	1.42	1.17	1	5
Lemon	3.99	2.06	1	7
Lime #	3.02	2.10	1	7
Loganberry #	2.51	2.45	1	7
Lychee	1.57	1.91	1	6
Mandarin ~	3.44	2.19	1	7
Mango #	2.43	2.40	1	7
Medlar	1.30	1.51	1	6
Melon	3.46	2.17	1	7
Nectarine ~	4.37	1.75	2	7
Nuts	5.63	1.46	3	7
Olive	2.96	2.48	1	7
Orange	5.91	1.27	3	7
Papaya	1.43	1.77	1	7
Passion fruit	2.41	2.57	1	7
Paw paw	1.38	2.06	1	7
Peach #	3.95	1.97	2	7
Pear	4.15	1.88	1	7
Pepper	4.81	1.76	2	7
Persimmon	1.29	1.20	1	5
Pineapple	3.64	2.21	1	7
Plantain	1.34	2.37	1	7
Plum	3.96	2.03	1	7
Pomelo	1.09	0.58	1	3
Pomegranate	2.45	2.25	1	7
Prune #	2.66	2.42	1	7
Quince #	1.86	1.77	1	7
Raisin	3.91	1.92	1	7
Rambotan	1.09	0.58	1	3

Fruit	Mean	SD	Range	
			min	max
Raspberry	5.19	1.62	3	7
Red currant	3.43	2.18	1	7
Redberry #	1.66	2.38	1	7
Red grape	3.76	2.08	1	7
Rhubarb	5.08	1.59	3	7
Rosehip #	2.50	2.48	1	7
Sapodilla	1.18	1.22	1	5
Satsuma ~	4.49	1.83	2	7
Sharon fruit	1.42	1.93	1	7
Sloe berry	1.71	2.37	1	7
Squash	2.03	2.44	1	7
Star fruit	1.55	1.95	1	7
Strawberry	5.82	1.04	4	7
Sultana	4.71	1.77	2	7
Tangelo *	1.24	1.98	1	7
Tangerine	4.17	2.05	2	7
Tayberry	1.47	1.81	1	7
Tomato	5.89	1.26	4	7
Walnut #	5.77	1.13	4	7
Water melon	3.30	2.19	1	7
Whinberry	1.34	0.99	1	4
White currant	1.64	2.10	1	7
White grape	3.21	2.32	1	7
Whortleberry	1.28	1.63	1	7

APPENDIX C

Table C.1a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to the 18-20 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	2.62	1.55	1	6
Adder	2.95	1.53	1	6
Albatross	2.23	1.59	1	6
Alligator	4.90	1.41	2	7
Alpaca #	1.88	1.71	1	7
Amoeba	1.16	1.50	1	7
Angel fish	1.77	1.01	1	4
Ant	2.14	2.08	1	7
Ant bear #	1.63	1.22	1	6
Anteater	2.61	1.52	1	7
Antelope #	2.79	1.83	1	7
Ape	4.98	1.46	1	7
Armadillo	2.22	1.53	1	6
Ass #	2.16	1.90	1	7
Baboon	3.82	1.68	1	7
Badger	4.49	1.31	2	7
Bald eagle	2.50	1.99	1	7
Bat	3.76	1.52	2	7
Bear	6.17	0.91	4	7
Beaver	3.44	1.60	1	7
Bee	2.89	2.00	1	7
Beetle	3.08	1.83	1	7
Bird #	4.35	1.86	1	7
Bison #	2.18	1.55	1	5
Blackbird	3.30	1.49	1	7
Blue bird	1.86	1.61	1	6
Blue tit	2.85	1.59	1	6
Boa constrictor	2.59	1.47	1	6
Boar #	3.49	1.39	1	7
Brontosaurus #	1.51	1.76	1	7
Brown bear	5.25	1.26	3	7
Buck #	1.79	1.94	1	7

Animal	Mean	SD	Range min	Range max
Budgerigar	1.82	1.88	1	7
Buffalo	3.52	1.89	1	7
Bull	4.53	1.51	2	7
Bullfinch # *	1.81	1.33	1	6
Bullock #	2.13	1.69	1	7
Butterfly	3.04	1.83	1	7
Buzzard	1.97	1.55	1	6
Calf ~	3.99	1.82	1	7
Camel #	4.09	1.49	1	7
Canary	2.65	1.61	1	7
Caribou #	1.58	1.75	1	7
Carp	2.44	1.39	1	7
Cat	6.70	0.51	5	7
Caterpillar	2.59	1.65	1	7
Cattle	4.85	1.61	2	7
Chaffinch	1.85	1.40	1	6
Chameleon	2.31	1.70	1	7
Cheetah	5.14	1.29	2	7
Chicken #	5.41	1.37	2	7
Chimpanzee	5.88	1.17	3	7
Chinchilla	2.53	1.37	1	5
Chipmunk	3.09	1.56	1	7
Clown fish	1.85	1.27	1	5
Cobra #	3.57	1.61	1	7
Cockatiel	2.18	1.92	1	7
Cocker spaniel ~	4.31	1.63	1	7
Cockerel	3.24	1.65	1	7
Cockroach	1.73	1.66	1	6
Cod	2.68	1.93	1	7
Condor #	1.60	1.41	1	6
Conger eel	1.39	1.19	1	5
Cougar #	2.27	2.08	1	7
Cow	6.22	0.92	3	7
Coyote	3.27	1.47	1	7
Cray fish	1.80	1.43	1	6
Crocodile	5.53	1.24	3	7
Crow	2.59	1.73	1	7
Cuckoo	2.18	1.47	1	6
Deer	5.25	1.20	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Dingo #	2.04	1.50	1	6
Dog #	6.85	0.34	6	7
Dolphin	5.25	1.37	2	7
Donkey	5.55	1.19	3	7
Dormouse	2.63	1.57	1	7
Dove	3.78	1.49	2	7
Dragon	1.83	2.10	1	7
Dragonfly	2.16	1.64	1	7
Dromedary #	1.15	1.10	1	5
Duck	4.80	1.54	2	7
Duckbill platypus	1.88	1.68	1	7
Eagle	4.89	1.35	2	7
Earthworm	1.96	1.97	1	7
Earwig	1.59	1.20	1	5
Echidna	1.21	1.14	1	5
Elephant	6.52	0.62	5	7
Eel	2.14	1.56	1	7
Elk #	1.85	1.71	1	7
Emu	2.53	1.77	1	7
Ewe #	2.97	1.48	1	6
Ferret	3.65	1.44	2	7
Field mouse	2.77	1.46	1	6
Finch	1.99	1.65	1	6
Fish #	4.12	1.92	1	7
Flamingo	3.44	1.39	1	7
Flea	1.59	1.86	1	7
Fly	2.19	2.27	1	7
Fowl	2.56	1.76	1	7
Fox	5.26	1.23	3	7
Frog #	4.02	1.74	1	7
Gazelle #	2.79	1.82	1	7
Gecko	1.80	1.59	1	7
Gerbil	3.14	1.85	1	7
Giant panda	5.00	1.24	3	7
Gibbon	1.96	2.11	1	7
Giraffe	6.10	0.84	4	7
Gnu #	1.13	1.18	1	5
Goldcrest	1.50	1.22	1	5
Goat	5.25	1.21	3	7

Animal	Mean	SD	Range min	max
Goldfish	3.41	1.99	1	7
Gorilla	3.38	1.55	1	7
Greyhound	5.93	0.96	3	7
Grizzly bear	4.93	1.41	2	7
Groundhog	5.65	1.17	2	7
Guinea fowl	2.32	1.76	1	7
Guinea pig	1.87	1.45	1	6
Gull	4.52	1.49	2	7
Haddock	2.14	1.82	1	7
Hamster	2.41	1.91	1	7
Hare	4.70	1.53	2	7
Hart #	3.45	1.51	1	7
Hawk	1.55	1.35	1	5
Hedgehog	3.16	1.85	1	7
Heifer #	4.31	1.51	2	7
Hen	1.36	1.63	1	6
Heron	4.19	1.62	2	7
Herring	2.55	1.84	1	7
Hippopotamus	2.08	1.64	1	6
Hornet	5.61	1.21	3	7
Horse	1.67	1.76	1	7
Horsefly	6.51	0.72	4	7
Hyena	1.57	1.33	1	6
Ibex #	3.59	1.46	1	7
Iguana	1.24	1.05	1	4
Impala #	3.11	1.37	1	6
Insect	1.38	1.78	1	7
Invertebrate	2.90	2.32	1	7
Jack rabbit	1.78	2.34	1	7
Jackal #	2.00	1.95	1	7
Jackass #	2.06	1.56	1	6
Jackdaw #	1.65	1.93	1	7
Jaguar	1.52	1.30	1	6
Kangaroo	4.62	1.47	2	7
Kid #	5.37	1.25	3	7
Kitten	1.83	1.71	1	7
Kiwi	5.22	1.29	1	7
Koala	1.65	1.88	1	7
Koi carp	4.19	1.36	2	7

Animal	Mean	SD	Range min	max
Komodo dragon	2.65	1.71	1	6
Lady bird	1.36	1.31	1	5
Lamb #	2.27	2.06	1	7
Lemur	4.92	1.32	3	7
Leopard	2.71	1.57	1	7
Lion	5.80	0.98	4	7
Lizard	6.63	0.64	4	7
Llama #	3.90	1.76	2	7
Lobster	3.19	1.57	1	7
Long tailed tit	2.76	1.88	1	7
Lynx	1.77	1.31	1	6
Mackerel	2.22	1.98	1	7
Mammal	2.21	1.46	1	6
Manatee	5.55	1.41	3	7
Mandrill # *	1.76	1.73	1	7
Marmoset #	1.40	1.37	1	6
Marmot #	1.30	1.04	1	5
Marten #	1.46	1.12	1	5
Meerkat	1.24	0.93	1	4
Midge	3.47	1.58	1	7
Mink #	1.38	1.21	1	5
Manx	1.86	1.43	1	6
Mole	2.35	1.48	1	6
Mongoose #	3.68	1.25	2	7
Monkey #	2.32	1.45	1	6
Moose	6.42	0.72	4	7
Moth	3.83	1.36	1	7
Mouse ~	1.97	1.72	1	7
Mule	3.78	1.89	1	7
Musk ox #	2.98	1.86	1	7
Newt	1.19	1.06	1	5
Nightingale	1.86	1.76	1	7
Ocelot #	1.95	1.61	1	7
Octopus	1.29	1.54	1	6
Orang-utan	3.93	1.70	1	7
Oryx #	3.76	1.80	1	7
Ostrich	1.36	1.52	1	6
Otter #	4.12	1.29	2	7
Owl	3.12	1.63	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Ox #	2.88	1.73	1	7
Panda	5.54	1.21	3	7
Panther	4.69	1.36	2	7
Parakeet	1.84	1.61	1	6
Parrot	4.26	1.62	2	7
Partridge	1.85	1.28	1	5
Peacock	3.33	11.77	1	6
Peewit #	1.13	1.25	1	6
Pelican	2.80	1.20	1	5
Penguin ~	4.78	1.50	2	7
Perch	1.59	1.35	1	5
Pheasant	3.30	1.43	1	7
Pig	5.87	1.12	2	7
Pigeon	3.76	1.90	2	7
Piglet ~	4.56	1.47	1	7
Pike	2.18	1.63	1	7
Pine marten #	1.31	1.06	1	4
Piranha fish	3.03	1.34	1	7
Plaice	1.98	1.60	1	7
Platypus	1.99	1.70	1	7
Polar bear	6.01	0.93	4	7
Polar cat	2.41	1.85	1	6
Pony	5.82	1.02	3	7
Porcupine	2.48	1.53	1	7
Porpoise #	1.66	1.89	1	7
Poultry	2.77	2.28	1	7
Prairie dog	2.67	1.92	1	7
Puffin	2.94	1.56	1	7
Puma	3.41	1.88	1	7
Rabbit	5.86	1.12	3	7
Racoon	2.92	1.48	1	7
Ram #	2.91	1.89	1	7
Rat	3.80	1.77	1	7
Raven	2.55	1.69	1	7
Reindeer	4.60	1.38	2	7
Rhesus monkey #	2.48	2.15	1	7
Rhinoceros	5.43	1.19	2	7
Roach	1.77	1.37	1	7
Robin	3.35	1.68	1	7

Animal	Mean	SD	Range min	max
Rodent	2.96	1.84	1	7
Roe dear #	1.44	1.68	1	6
Rook	1.63	1.32	1	5
Rooster	3.03	1.63	1	7
Salamander	2.20	1.70	1	7
Salmon	2.85	1.68	1	7
Sardine	2.02	1.57	1	6
Sea lion	3.16	1.60	1	7
Seagull	3.22	1.87	1	7
Seahorse	2.39	1.35	1	6
Seal	4.07	1.49	1	7
Shark #	5.59	1.28	3	7
Sheep	5.86	1.08	3	7
Short tailed tit	1.60	1.13	1	5
Shrew	2.10	1.59	1	7
Shrimp	1.98	1.47	1	6
Siamese cat ~	3.98	1.61	2	7
Siberian tiger	3.78	1.85	1	7
Skate	1.55	1.22	1	5
Skunk	3.36	1.30	1	6
Skylark #	1.78	1.24	1	5
Sloth	2.99	1.71	1	7
Slug	1.88	1.87	1	7
Snail	2.42	1.67	1	6
Snake #	4.87	1.69	2	7
Sole	1.61	1.33	1	5
Sow #	1.70	1.78	1	6
Sparrow	2.94	1.73	1	7
Spider	3.26	2.04	1	7
Springbok #	1.71	1.77	1	7
Squid	2.44	1.62	1	7
Squirrel	4.73	1.38	3	7
Stag	3.23	1.93	1	7
Star fish	1.94	1.42	1	6
Starling	2.08	1.74	1	7
Stick insect	1.90	1.79	1	7
Stickleback	1.91	1.08	1	5
Stoat #	2.05	1.51	1	6
Sturgeon	1.45	1.62	1	6

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Swan	3.81	1.58	1	7
Swift #	1.55	1.56	1	6
Sword fish	2.91	1.67	1	7
Tapir #	1.48	1.83	1	7
Tarantula #	3.29	1.81	1	7
Tench	1.40	1.17	1	4
Thrush	2.17	1.54	1	7
Tiger	6.56	0.56	5	7
Toad	3.24	1.66	1	7
Tortoise	4.78	1.42	3	7
Trout	2.53	1.90	1	7
Tuna	2.51	1.65	1	7
Turkey	4.44	1.52	2	7
Turtle	4.64	1.44	2	7
Tyrannosaurus	1.64	2.08	1	7
Vole	2.45	1.42	1	6
Vulture	2.42	1.73	1	7
Wallaby #	2.11	1.73	1	7
Walrus	3.29	1.71	1	7
Warthog	2.43	1.76	1	7
Wasp	2.37	2.21	1	7
Water buffalo #	2.96	1.49	1	7
Water rat	2.51	1.33	1	6
Weasel	3.37	1.38	1	7
Whale	5.56	1.33	3	7
White tiger	4.58	1.43	2	7
Wild boar	2.65	1.72	1	7
Wild cat	3.56	1.83	1	7
Wild dog ~	3.35	1.69	1	7
Wildebeest #	2.82	2.00	1	7
Wolf	5.45	1.23	3	7
Wombat	2.69	1.47	1	7
Woodcock	1.61	1.31	1	5
Woodlouse	1.80	1.72	1	5
Woodpecker	3.14	1.54	1	7
Worm	2.25	2.14	1	7
Wren	2.09	1.51	1	6
Yak #	2.18	1.51	1	7
Yellow tit	1.83	1.29	1	5

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	**Mean**	**SD**	**Range** min	max
Yellowhammer #	1.30	1.68	1	7
Yorkshire terrier	4.22	1.75	1	7
Zebra	5.89	0.96	4	7

Table C.1b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to the 18-20 age –category.

Fruit	**Mean**	**SD**	**Range** min	max
Acorn	1.50	1.54	1	6
Almond	2.10	1.49	1	6
Apple	6.89	0.30	6	7
Apricot	5.06	1.25	2	7
Aubergine #	2.00	1.64	1	7
Avocado #	2.64	1.81	1	7
Banana	6.89	0.30	6	7
Berries	5.30	1.37	3	7
Bilberry #	1.27	1.57	1	7
Blackberry	5.19	1.00	3	7
Blackcurrant	4.41	1.48	1	7
Blueberry	4.35	1.46	2	7
Bramble #	1.88	1.93	1	7
Butternut squash	1.72	1.36	1	5
Cantaloupe melon	3.01	1.81	1	7
Cherry	5.48	1.27	2	7
Chestnut #	1.97	1.63	1	6
Citron	2.03	2.16	1	7
Clementine ~	4.63	1.45	2	7
Coconut	3.81	1.83	1	7
Cox apple # *	3.73	1.70	1	7
Crab apple	2.34	1.71	1	7
Cranberry	4.13	1.55	2	7
Cucumber #	2.00	1.87	1	7
Currant #	3.19	1.77	1	7
Damson #	1.45	1.64	1	6
Date	2.65	1.33	1	6
Dewberry #	1.93	1.63	1	7
Elderberry #	2.34	1.77	1	7
Fig #	2.50	1.49	1	7
Gala apple *	4.12	1.74	1	7
Galia melon # ~	2.46	2.09	1	7
Gooseberry #	2.94	1.68	1	7
Granny smith	4.07	1.94	1	7
Grapefruit	4.97	1.31	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	Range max
Grape	6.24	0.96	3	7
Green melon	3.65	1.88	1	7
Guava	1.92	1.84	1	7
Haw	1.11	0.99	1	5
Hazelnuts	2.30	1.54	1	6
Honeydew melon # ~	3.44	1.76	1	7
Horse chestnut	1.79	1.71	1	6
Jackfruit	1.47	1.51	1	6
Jaffa #	1.38	1.91	1	7
Kiwi	4.72	1.41	2	7
kumquat	1.71	1.50	1	7
Lemon	4.58	1.45	1	7
Lime	4.17	1.64	1	7
Loganberry #	1.68	1.92	1	7
Lychee # ~	1.94	1.58	1	6
Mandarin # ~	3.90	1.47	1	7
Mango	5.22	1.29	3	7
Melon	5.55	1.34	3	8
Nashi	1.10	0.92	1	5
Nectarine	5.27	1.33	3	7
Nuts # ~	2.18	2.04	1	7
Olive #	1.97	1.85	1	7
Orange	5.73	1.14	1	7
Papaya	2.37	1.89	1	7
Passion fruit	4.15	1.33	1	7
Paw paw #	1.30	1.56	1	6
Peach ~	5.92	0.89	4	7
Pear	5.98	1.05	4	7
Peppers	2.26	1.96	1	7
Persimmons	1.18	1.12	1	5
Pineapple	5.54	1.29	2	7
Plantain #	1.43	1.40	1	5
Plum	5.26	1.23	3	7
Pomelo	1.31	1.26	1	5
Pomegranate ~	3.25	1.69	1	7
Prune #	2.33	1.64	1	6
Quince	1.14	1.08	1	5
Raisin #	2.99	1.93	1	7
Raspberry	5.38	1.22	3	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	Range max
Red currant #	2.66	1.67	1	7
Redberry	3.00	1.63	1	7
Red grape	4.03	1.83	1	7
Rhubarb #	3.03	1.64	1	7
Rosehip # *	1.21	0.95	1	5
Satsuma ~	5.61	1.01	4	7
Sharon fruit ~	1.55	1.69	1	6
Sloe berry #	1.24	1.55	1	7
squash	2.09	1.63	1	6
Star fruit	2.14	1.77	1	7
Strawberry	6.51	0.72	4	7
Sultana	3.25	1.51	1	7
Tangerine	5.60	0.98	3	7
Tayberry	1.41	1.86	1	8
Tomato # *	2.96	2.13	1	7
Walnut #	1.90	1.45	1	6
Water melon	5.29	1.35	3	7
Whinberry	1.43	1.41	1	6
White currant #	1.93	1.61	1	7
White grape	3.00	2.25	1	7
Whortleberry	1.34	1.67	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.2a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to the 21-30 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	2.57	2.06	1	7
Adder	2.41	2.09	1	7
Albatross	2.47	1.76	1	7
Alligator	3.75	1.90	1	7
Alpaca #	1.65	2.21	1	7
Amoeba	1.31	1.81	1	7
Angel fish	1.62	1.51	1	6
Ant	2.00	2.49	1	7
Ant bear #	2.00	2.05	1	7
Anteater	2.92	1.87	1	7
Antelope #	3.25	2.02	1	7
Ape	5.32	1.34	1	7
Armadillo	2.39	2.06	1	7
Ass #	3.10	2.05	1	7
Baboon	4.19	1.65	1	7
Badger	4.79	1.46	1	7
Bald eagle	2.69	1.81	1	7
Bat	3.12	2.01	1	7
Bear	5.52	1.22	1	7
Beaver	4.26	1.72	2	7
Bee	2.57	2.14	1	7
Beetle	2.16	2.14	1	7
Bird #	3.40	2.11	1	7
Bison #	2.47	2.09	1	7
Blackbird	2.52	2.10	1	7
Blue bird	2.33	1.57	1	6
Blue tit	2.27	1.63	1	7
Boa constrictor	2.42	1.94	1	7
Boar #	3.62	1.88	1	7
Brontosaurus #	1.63	1.98	1	7
Brown bear	5.13	1.46	2	7
Buck #	1.98	2.01	1	7
Budgerigar	2.07	1.86	1	7
Buffalo	4.18	1.57	1	7
Bull	5.82	1.13	3	7

Animal	Mean	SD	Range min	max
Bullfinch # *	1.84	1.43	1	6
Bullock #	2.91	2.26	1	7
Butterfly	2.30	2.26	1	7
Buzzard	2.07	1.94	1	7
Calf ~	4.96	1.54	2	7
Camel #	4.83	1.60	2	7
Canary	2.51	1.86	1	7
Caribou #	2.11	2.31	1	7
Carp	1.90	1.69	1	7
Cat	5.91	1.27	2	7
Caterpillar	1.97	2.10	1	7
Cattle	5.30	1.48	3	7
Chaffinch	1.78	1.43	1	6
Chameleon	2.47	1.74	1	7
Cheetah	5.01	1.40	1	7
Chicken #	4.56	1.79	1	7
Chimpanzee	6.01	1.03	3	7
Chinchilla	3.12	1.91	1	7
Chipmunk	3.58	1.78	1	7
Clown fish	1.98	1.58	1	6
Cobra #	2.85	1.86	1	7
Cockatiel	2.32	1.82	1	7
Cocker spaniel ~	3.85	1.95	1	7
Cockerel	3.01	1.96	1	7
Cockroach	1.82	2.21	1	7
Cod	2.28	2.19	1	7
Condor #	1.72	1.82	1	7
Conger eel	1.94	1.74	1	7
Cougar #	2.86	2.02	1	7
Cow	6.48	0.79	4	7
Coyote	2.84	1.90	1	7
Cray fish	1.69	1.37	1	7
Crocodile	4.03	1.72	1	7
Crow	3.18	1.98	1	7
Cuckoo	2.35	1.80	1	7
Deer	5.20	1.39	2	7
Dingo #	2.66	1.93	1	7
Dog #	6.62	0.72	4	7
Dolphin	4.16	1.75	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Donkey	5.43	1.42	2	7
Dormouse	3.38	1.67	1	7
Dove	2.76	1.89	1	7
Dragon	1.53	2.12	1	7
Dragonfly	1.97	1.93	1	7
Dromedary #	1.23	1.39	1	5
Duck	3.71	2.02	1	7
Duckbill platypus	2.38	1.69	1	7
Eagle	3.23	1.95	1	7
Earthworm	1.90	2.14	1	7
Earwig	1.65	1.92	1	7
Echidna	1.38	1.68	1	6
Elephant	2.08	1.94	1	7
Eel	5.45	1.31	1	7
Elk #	2.36	2.05	1	7
Emu	2.36	1.48	1	7
Ewe #	2.68	2.28	1	7
Ferret	3.83	1.77	1	7
Field mouse	3.79	1.75	1	7
Finch	2.16	1.50	1	6
Fish #	2.79	2.43	1	7
Flamingo	2.74	1.86	1	7
Flea	1.66	2.12	1	7
Fly	1.71	2.53	1	7
Fowl	2.46	1.79	1	7
Fox	5.80	1.12	3	7
Frog #	3.40	1.91	1	7
Gazelle #	2.44	2.28	1	7
Gecko	2.15	1.83	1	7
Gerbil	4.43	1.55	1	7
Giant panda	4.88	1.43	1	7
Gibbon	2.45	2.35	1	7
Giraffe	4.81	1.65	1	7
Gnu #	1.95	1.92	1	7
Goldcrest	4.99	1.51	1	7
Goat	1.52	1.50	1	6
Goldfish	2.36	2.46	1	7
Gorilla	3.24	1.91	1	7
Greyhound	5.98	1.11	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Grizzly bear	5.84	1.09	4	7
Groundhog	2.68	1.66	1	7
Guinea fowl	2.24	1.74	1	7
Guinea pig	4.65	1.43	1	7
Gull	2.26	1.57	1	7
Haddock	2.25	2.13	1	7
Hamster	5.23	1.25	2	7
Hare	4.71	1.41	2	7
Hart #	1.62	1.97	1	7
Hawk	2.95	1.96	1	7
Hedgehog	4.39	1.67	2	7
Heifer #	1.93	2.23	1	7
Hen	3.49	1.88	1	7
Heron	2.44	1.88	1	7
Herring	2.10	1.96	1	7
Hippopotamus	5.27	1.36	1	7
Hornet	1.68	1.56	1	6
Horse	6.20	1.00	3	7
Horsefly	1.72	1.34	1	6
Hyena	4.05	1.75	1	7
Ibex #	1.25	1.93	1	6
Iguana	2.17	1.78	1	7
Impala #	1.67	2.26	1	7
Insect	2.23	2.42	1	7
Invertebrate	1.80	2.15	1	7
Jack rabbit	2.79	2.07	1	7
Jackal #	1.96	2.12	1	7
Jackass #	1.72	1.92	1	7
Jackdaw #	1.80	1.56	1	6
Jaguar	5.17	1.34	2	7
Kangaroo	4.88	1.64	2	7
Kid #	2.74	2.06	1	7
Kitten	5.88	1.22	3	7
Kiwi	1.86	1.80	1	7
Koala	4.08	1.75	1	7
Koi carp	1.77	1.53	1	7
Komodo dragon	2.30	1.85	1	7
Lady bird	1.38	1.41	1	6
Lamb #	2.28	2.20	1	7

Animal	Mean	SD	Range min	Range max
Lemur	2.70	2.00	1	7
Leopard	5.47	1.28	2	7
Lion	6.38	0.76	4	7
Lizard	3.21	1.94	1	7
Llama #	3.25	1.96	1	7
Lobster	2.17	2.20	1	7
Long tailed tit	1.85	1.77	1	7
Lynx	2.96	1.84	1	7
Mackerel	2.09	1.78	1	7
Mammal	5.96	1.20	3	7
Manatee	2.00	1.77	1	7
Mandrill # *	1.66	1.76	1	6
Marmoset #	1.75	1.70	1	6
Marmot #	1.59	1.57	1	5
Marten #	1.49	1.11	1	4
Meerkat	3.10	1.91	1	7
Midge	1.43	1.98	1	7
Mink #	2.71	1.80	1	7
Manx	2.33	2.03	1	7
Mole	3.30	1.84	1	7
Mongoose #	2.39	1.68	1	7
Monkey #	6.31	0.79	4	7
Moose	4.22	1.87	2	8
Moth	2.10	2.15	1	7
Mouse ~	4.37	1.74	1	7
Mule	3.75	1.63	1	7
Musk ox #	1.33	1.43	1	5
Newt	1.93	1.87	1	7
Nightingale	2.06	1.69	1	7
Ocelot #	1.33	1.59	1	7
Octopus	2.59	2.03	1	7
Orang-utan	4.82	1.43	1	7
Oryx #	1.46	1.28	1	5
Ostrich	3.12	1.80	1	7
Otter #	3.66	1.76	1	7
Owl	3.62	1.87	1	7
Ox #	3.71	1.89	1	7
Panda	5.79	1.16	3	7
Panther	5.11	1.30	2	7

~ significant effect of age, **#** significant effect of gender, ***** significant effect of education

Animal	Mean	SD	Range min	Range max
Parakeet	1.92	1.62	1	6
Parrot	3.36	1.94	1	7
Partridge	2.32	1.81	1	7
Peacock	3.20	1.72	1	7
Peewit #	1.19	0.85	1	4
Pelican	2.73	1.85	1	7
Penguin ~	4.16	1.80	1	7
Perch	1.65	1.41	1	6
Pheasant	3.06	1.83	1	7
Pig	6.23	0.93	4	7
Pigeon	3.02	2.09	1	7
Piglet ~	4.66	1.54	1	7
Pike	1.96	1.63	1	7
Pine marten #	1.72	1.33	1	5
Piranha fish	2.19	1.79	1	7
Plaice	1.94	1.68	1	7
Platypus	2.05	1.97	1	7
Polar bear	4.99	1.46	1	7
Polar cat	3.24	1.71	1	7
Pony	6.20	0.93	3	7
Porcupine	2.99	1.86	1	7
Porpoise #	2.20	1.69	1	6
Poultry	2.98	2.12	1	7
Prairie dog	2.79	1.98	1	7
Puffin	2.81	1.91	1	7
Puma	4.28	1.50	1	7
Rabbit	6.01	1.12	2	7
Racoon	3.25	1.92	1	7
Ram #	3.58	1.77	1	7
Rat	4.73	1.56	2	7
Raven	2.48	1.82	1	6
Reindeer	5.23	1.25	2	7
Rhesus monkey #	3.25	1.99	1	7
Rhinoceros	5.60	1.23	3	7
Roach	1.47	1.60	1	7
Robin	2.62	2.14	1	7
Rodent	3.66	1.94	1	7
Roe dear #	1.86	2.46	1	7
Rook	2.01	1.90	1	7

Animal	Mean	SD	Range min	max
Rooster	2.83	1.98	1	7
Salamander	2.01	1.78	1	7
Salmon	2.11	1.95	1	7
Sardine	1.90	2.14	1	7
Sea lion	3.21	1.80	1	7
Seagull	2.89	2.08	1	7
Seahorse	2.28	1.67	1	7
Seal	4.14	1.58	1	7
Shark #	3.62	1.84	1	7
Sheep	5.90	1.22	3	7
Short tailed tit	1.91	1.83	1	7
Shrew	2.15	1.78	1	7
Shrimp	1.96	2.08	1	7
Siamese cat ~	4.34	1.72	1	7
Siberian tiger	4.12	1.93	1	7
Skate	1.71	1.67	1	6
Skunk	3.15	1.93	1	7
Skylark #	1.75	1.34	1	5
Sloth	2.72	2.02	1	7
Slug	1.70	2.42	1	7
Snail	2.31	2.25	1	7
Snake #	3.56	1.86	1	7
Sole	1.53	1.43	1	7
Sow #	2.35	2.34	1	7
Sparrow	2.70	1.92	1	7
Spider	2.54	2.42	1	7
Springbok #	2.06	2.10	1	7
Squid	1.96	1.99	1	7
Squirrel	5.21	1.33	2	7
Stag	3.83	1.79	1	7
Star fish	1.95	2.00	1	7
Starling	2.09	2.05	1	7
Stick insect	1.79	2.04	1	7
Stickleback	1.85	2.05	1	7
Stoat #	2.35	2.06	1	7
Sturgeon	1.56	1.52	1	7
Swan	3.34	1.90	1	7
Swift #	1.59	1.51	1	6
Sword fish	2.05	1.67	1	7

Animal	Mean	SD	Range min	max
Tapir #	1.58	1.71	1	6
Tarantula #	2.37	2.31	1	7
Tench	1.51	1.37	1	5
Thrush	2.03	1.71	1	6
Tiger	6.05	1.07	3	7
Toad	2.95	1.98	1	7
Tortoise	3.30	1.80	1	7
Trout	2.13	1.96	1	7
Tuna	2.08	2.06	1	7
Turkey	3.59	1.80	1	7
Turtle	3.35	1.77	1	7
Tyrannosaurus	1.91	2.45	1	7
Vole	2.35	2.16	1	7
Vulture	2.72	1.75	1	7
Wallaby #	2.74	1.98	1	7
Walrus	2.92	1.92	1	7
Warthog	2.60	1.94	1	7
Wasp	2.30	2.27	1	7
Water buffalo #	3.02	2.02	1	7
Water rat	2.44	1.76	1	7
Weasel	3.05	2.00	1	7
Whale	4.06	1.91	1	7
White tiger	4.20	1.78	1	7
Wild boar	4.19	1.53	1	7
Wild cat	3.32	1.94	1	7
Wild dog ~	3.78	1.71	1	7
Wildebeest #	3.62	1.84	1	7
Wolf	5.86	1.11	3	7
Wombat	2.23	1.77	1	7
Woodcock	1.95	1.55	1	6
Woodlouse	1.57	1.98	1	7
Woodpecker	2.55	1.87	1	7
Worm	1.95	2.14	1	7
Wren	1.87	1.68	1	7
Yak #	2.42	2.25	1	7
Yellow tit	1.83	1.17	1	5
Yellowhammer #	1.43	1.50	1	7
Yorkshire terrier	4.36	1.70	1	7
Zebra	4.98	1.55	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Table C.2b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to the 21-30 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	1.40	1.82	1	7
Almond	1.66	1.96	1	7
Apple	5.85	1.07	1	7
Apricot	4.89	1.40	1	7
Aubergine #	2.40	2.00	1	7
Avocado #	2.51	2.05	1	7
Banana	6.35	1.03	3	7
Berries	4.65	1.54	1	7
Bilberry #	1.53	1.65	1	7
Blackberry	4.51	1.68	1	7
Blackcurrant	4.94	1.60	2	7
Blueberry	4.04	1.76	1	7
Bramble #	2.86	2.02	1	7
Butternut squash	1.72	2.06	1	7
Cantaloupe melon	2.60	2.23	1	7
Cherry	4.79	1.60	1	7
Chestnut #	1.69	1.46	1	6
Citron	2.42	2.18	1	7
Clementine ~	4.07	1.76	1	7
Coconut	3.72	1.96	1	7
Cox apple # *	4.63	1.74	1	7
Crab apple	4.17	1.56	1	7
Cranberry	4.14	1.71	1	7
Cucumber #	1.97	2.17	1	7
Currant #	3.47	1.80	1	7
Damson #	1.90	1.71	1	6
Date	2.86	1.93	1	7
Dewberry #	1.90	1.90	1	7
Elderberry #	2.62	1.82	1	7
Fig #	2.96	1.72	1	7
Gala apple *	3.92	1.90	1	7
Galia melon # ~	3.68	1.86	1	7
Gooseberry #	3.34	1.84	1	7
Granny smith	5.34	1.41	2	7
Grapefruit	5.10	1.48	2	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Fruit	Mean	SD	Range min	Range max
Grape	6.37	0.94	3	7
Green melon	3.31	2.08	1	7
Guava	1.77	2.02	1	7
Haw	1.24	1.31	1	5
Hazelnuts	1.59	1.87	1	7
Honeydew melon # ~	4.27	1.68	1	7
Horse chestnut	1.60	1.72	1	7
Jackfruit	1.57	2.06	1	7
Jaffa #	2.33	2.13	1	7
Kiwi	4.70	1.66	2	7
kumquat	2.05	2.07	1	7
Lemon	5.30	1.52	2	7
Lime	4.41	1.70	1	7
Loganberry #	2.05	1.87	1	7
Lychee # ~	2.41	2.03	1	7
Mandarin # ~	4.39	1.70	1	7
Mango	4.01	1.83	1	7
Melon	5.78	1.25	2	7
Nashi	1.16	1.00	1	5
Nectarine	4.88	1.52	1	7
Nuts # ~	1.68	2.08	1	7
Olive #	1.84	2.25	1	7
Orange	6.86	0.33	6	7
Papaya	3.27	1.72	1	7
Passion fruit	4.40	1.48	1	7
Paw paw #	1.62	2.13	1	7
Peach ~	5.54	1.17	1	7
Pear	6.07	1.09	2	7
Peppers	1.97	2.46	1	7
Persimmons	1.36	1.58	1	7
Pineapple	5.60	1.33	2	7
Plantain #	1.50	1.84	1	7
Plum	5.01	1.52	1	7
Pomelo	1.31	1.31	1	5
Pomegranate ~	3.50	1.73	1	7
Prune #	2.78	1.89	1	7
Quince	1.36	1.13	1	4
Raisin #	3.09	2.08	1	7
Raspberry	5.37	1.39	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	Range max
Red currant #	2.95	1.70	1	7
Redberry	2.55	2.06	1	7
Red grape	5.13	1.46	2	7
Rhubarb #	3.15	2.19	1	7
Rosehip # *	1.47	1.66	1	7
Satsuma ~	4.89	1.55	1	7
Sharon fruit ~	2.03	2.07	1	7
Sloe berry #	2.12	1.78	1	7
squash	1.91	1.92	1	7
Star fruit	2.25	2.31	1	7
Strawberry	6.49	0.82	3	7
Sultana	2.94	1.99	1	7
Tangerine	5.68	1.23	2	7
Tayberry	1.44	1.26	1	5
Tomato # *	2.95	2.32	1	7
Walnut #	1.64	1.75	1	7
Water melon	5.73	1.19	2	7
Whinberry	1.76	1.97	1	7
White currant #	1.78	1.85	1	7
White grape	4.86	1.37	1	7
Whortleberry	1.41	1.79	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.3a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to the 31-40 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark	2.14	2.25	1	7
Adder	2.74	1.98	1	7
Albatross	2.93	1.59	1	7
Alligator	4.21	1.65	2	7
Alpaca #	1.92	2.05	1	7
Amoeba	1.38	1.64	1	7
Angel fish	1.90	1.69	1	6
Ant	2.03	2.23	1	7
Ant bear #	2.00	2.15	1	7
Anteater	3.36	1.72	1	7
Antelope #	3.98	1.65	1	7
Ape	4.71	1.68	1	7
Armadillo	2.65	1.85	1	7
Ass #	3.72	1.66	1	7
Baboon	4.09	1.74	1	7
Badger	5.29	1.29	2	7
Bald eagle	2.57	2.11	1	7
Bat	2.79	2.15	1	7
Bear	3.96	2.02	1	7
Beaver	4.37	1.61	2	7
Bee	2.32	2.14	1	7
Beetle	1.85	1.85	1	6
Bird #	2.89	2.37	1	7
Bison #	2.81	2.04	1	7
Blackbird	3.05	2.05	1	7
Blue bird	2.47	2.19	1	7
Blue tit	3.04	2.09	1	7
Boa constrictor	2.21	1.95	1	6
Boar #	3.84	1.76	1	7
Brontosaurus #	2.49	2.05	1	7
Brown bear	5.30	1.39	2	7
Buck #	2.48	2.11	1	7
Budgerigar	2.60	1.92	1	7
Buffalo	4.50	1.47	1	7
Bull	4.61	1.62	1	7

Animal	Mean	SD	Range min	max
Bullfinch # *	2.08	1.93	1	7
Bullock #	3.49	1.92	1	7
Butterfly	2.84	2.13	1	7
Buzzard	2.37	1.88	1	7
Calf ~	4.27	1.76	1	7
Camel #	5.71	1.24	2	7
Canary	2.45	1.67	1	6
Caribou #	2.35	2.16	1	7
Carp	2.11	2.11	1	7
Cat	5.32	1.35	1	7
Caterpillar	1.90	1.79	1	7
Cattle	6.24	0.91	4	7
Chaffinch	2.09	2.12	1	7
Chameleon	3.28	1.63	1	7
Cheetah	5.20	1.51	2	7
Chicken #	4.35	1.88	1	7
Chimpanzee	4.90	1.64	1	7
Chinchilla	2.63	1.95	1	7
Chipmunk	3.59	2.02	1	7
Clown fish	1.93	1.88	1	6
Cobra #	2.91	1.84	1	7
Cockatiel	2.77	1.84	1	7
Cocker spaniel ~	5.63	1.21	4	7
Cockerel	3.33	1.79	1	7
Cockroach	2.37	1.75	1	7
Cod	2.35	2.06	1	7
Condor #	2.16	1.66	1	7
Conger eel	1.99	1.69	1	6
Cougar #	3.32	1.95	1	7
Cow	6.22	1.12	2	7
Coyote	4.20	1.64	1	7
Cray fish	2.06	1.72	1	7
Crocodile	4.31	1.71	2	7
Crow	2.94	2.15	1	7
Cuckoo	2.46	2.09	1	7
Deer	4.68	1.60	1	7
Dingo #	2.84	2.11	1	7
Dog #	5.38	1.34	1	7
Dolphin	4.76	1.53	2	7

Animal	Mean	SD	Range min	max
Donkey	5.12	1.34	1	7
Dormouse	2.96	1.88	1	7
Dove	3.01	1.77	1	7
Dragon	1.41	1.83	1	7
Dragonfly	2.28	2.01	1	7
Dromedary #	2.03	2.14	1	7
Duck	3.85	1.94	1	7
Duckbill platypus	2.17	1.70	1	6
Eagle	2.99	2.14	1	7
Earthworm	1.90	2.24	1	7
Earwig	1.52	1.19	1	5
Echidna	1.47	1.84	1	6
Elephant	2.37	2.08	1	7
Eel	5.14	1.47	1	7
Elk #	2.94	1.92	1	7
Emu	3.09	1.80	1	7
Ewe #	5.68	1.03	4	7
Ferret	4.18	1.48	2	7
Field mouse	3.27	2.10	1	7
Finch	2.85	1.99	1	7
Fish #	2.72	2.35	1	7
Flamingo	2.37	2.06	1	7
Flea	1.54	1.63	1	6
Fly	1.92	2.44	1	7
Fowl	2.42	1.90	1	7
Fox	5.04	1.66	2	7
Frog #	3.64	2.00	1	7
Gazelle #	4.43	1.73	2	7
Gecko	2.32	1.49	1	6
Gerbil	4.16	1.80	2	7
Giant panda	4.24	1.95	1	7
Gibbon	4.82	1.40	3	7
Giraffe	6.10	1.04	3	7
Gnu #	2.40	1.91	1	7
Goldcrest	4.56	1.77	1	7
Goat	1.70	1.76	1	6
Goldfish	2.45	2.32	1	7
Gorilla	3.94	1.80	2	7
Greyhound	5.69	1.33	3	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Grizzly bear	5.23	1.35	3	7
Groundhog	2.22	1.99	1	7
Guinea fowl	2.22	1.77	1	6
Guinea pig	4.54	1.52	2	7
Gull	2.71	2.07	1	7
Haddock	2.71	2.16	1	7
Hamster	5.14	1.50	3	7
Hare	4.99	1.40	2	7
Hart #	1.43	1.78	1	6
Hawk	2.61	1.96	1	7
Hedgehog	4.76	1.39	3	7
Heifer #	3.14	1.94	1	7
Hen	3.52	2.16	1	7
Heron	2.45	1.90	1	7
Herring	2.68	1.87	1	7
Hippopotamus	5.58	1.23	2	7
Hornet	1.85	1.47	1	6
Horse	5.38	1.34	1	7
Horsefly	1.59	1.48	1	6
Hyena	3.89	1.86	1	7
Ibex #	1.64	1.95	1	7
Iguana	3.03	1.80	1	7
Impala #	2.41	2.09	1	7
Insect	2.03	2.37	1	7
Invertebrate	2.04	1.98	1	7
Jack rabbit	3.72	1.67	2	7
Jackal #	2.82	2.04	1	7
Jackass #	2.74	1.67	1	7
Jackdaw #	2.13	2.19	1	7
Jaguar	4.45	1.67	1	7
Kangaroo	5.24	1.39	3	7
Kid #	3.05	1.98	1	7
Kitten	5.17	1.39	1	7
Kiwi	2.26	1.99	1	7
Koala	5.12	1.31	3	7
Koi carp	2.23	1.86	1	7
Komodo dragon	2.17	2.14	1	7
Lady bird	1.36	1.32	1	6
Lamb #	2.07	2.16	1	7

Animal	Mean	SD	Range min	max
Lemur	2.91	1.98	1	7
Leopard	4.97	1.57	2	7
Lion	6.13	1.14	2	7
Lizard	2.35	2.13	1	7
Llama #	2.99	2.11	1	7
Lobster	1.97	1.81	1	6
Long tailed tit	1.86	2.09	1	7
Lynx	3.77	1.70	1	7
Mackerel	2.54	1.82	1	7
Mammal	4.45	1.71	1	7
Manatee	2.13	2.29	1	7
Mandrill # *	1.46	1.30	1	5
Marmoset #	2.02	1.94	1	7
Marmot #	1.87	1.86	1	7
Marten #	2.01	1.49	1	6
Meerkat	4.41	1.34	2	7
Midge	1.52	1.88	1	7
Mink #	3.20	1.86	1	7
Manx	2.95	2.26	1	7
Mole	3.78	1.82	2	7
Mongoose #	2.55	2.14	1	7
Monkey #	4.83	1.65	1	7
Moose	3.99	1.77	1	7
Moth	2.18	2.30	1	7
Mouse ~	3.79	2.04	1	7
Mule	3.85	1.85	1	7
Musk ox #	2.47	2.30	1	7
Newt	1.87	1.68	1	6
Nightingale	2.58	2.13	1	7
Ocelot #	1.41	1.30	1	4
Octopus	3.73	1.70	2	7
Orang-utan	5.51	1.39	3	7
Oryx #	1.53	1.83	1	6
Ostrich	3.47	1.82	1	7
Otter #	4.48	1.57	2	7
Owl	2.95	2.14	1	7
Ox #	3.98	1.80	1	7
Panda	5.32	1.57	2	7
Panther	3.82	1.93	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Parakeet	2.29	1.80	1	6
Parrot	3.07	2.06	1	7
Partridge	2.41	1.85	1	7
Peacock	4.02	1.57	2	7
Peewit #	1.23	1.87	1	7
Pelican	2.32	2.11	1	7
Penguin ~	4.33	1.76	1	7
Perch	1.98	1.85	1	7
Pheasant	2.95	1.98	1	7
Pig	5.20	1.39	1	7
Pigeon	2.89	2.28	1	7
Piglet ~	5.38	1.26	3	7
Pike	2.38	1.84	1	6
Pine marten #	1.92	1.76	1	7
Piranha fish	2.15	1.77	1	6
Plaice	2.28	1.78	1	7
Platypus	2.79	1.81	1	7
Polar bear	5.43	1.38	2	7
Polar cat	2.47	2.13	1	7
Pony	4.86	1.49	1	7
Porcupine	4.00	1.58	2	7
Porpoise #	2.30	1.88	1	7
Poultry	2.78	2.09	1	7
Prairie dog	3.99	1.71	2	7
Puffin	3.01	1.73	2	7
Puma	3.37	2.00	1	7
Rabbit	4.86	1.59	1	7
Racoon	3.41	1.87	1	7
Ram #	3.91	1.73	1	7
Rat	3.60	2.02	1	7
Raven	2.98	1.76	1	7
Reindeer	5.31	1.36	2	7
Rhesus monkey #	2.48	2.52	1	7
Rhinoceros	5.55	1.21	3	7
Roach	1.71	1.46	1	6
Robin	3.39	1.92	1	7
Rodent	2.73	2.10	1	7
Roe dear #	2.48	2.33	1	7
Rook	2.83	1.80	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Rooster	3.37	1.85	1	7
Salamander	1.71	1.38	1	6
Salmon	2.54	1.92	1	7
Sardine	2.02	1.37	1	6
Sea lion	3.22	2.01	1	7
Seagull	3.26	2.05	1	7
Seahorse	1.88	1.71	1	7
Seal	3.74	1.89	1	7
Shark #	3.46	1.76	1	7
Sheep	5.24	1.40	1	7
Short tailed tit	1.76	1.82	1	6
Shrew	2.60	1.69	1	7
Shrimp	1.83	1.89	1	7
Siamese cat ~	3.54	1.99	1	7
Siberian tiger	3.53	1.95	1	7
Skate	2.22	1.31	1	5
Skunk	3.97	1.47	2	7
Skylark #	2.08	2.02	1	7
Sloth	3.14	1.96	1	7
Slug	1.87	2.00	1	7
Snail	2.02	2.26	1	7
Snake #	2.87	2.45	1	7
Sole	2.07	1.26	1	5
Sow #	3.49	1.94	1	7
Sparrow	2.60	2.26	1	7
Spider	2.08	2.48	1	7
Springbok #	2.89	1.73	1	7
Squid	2.50	1.95	1	7
Squirrel	5.53	1.27	3	7
Stag	4.60	1.69	2	7
Star fish	1.59	1.44	1	6
Starling	2.35	1.99	1	7
Stick insect	1.92	1.39	1	5
Stickleback	1.90	1.54	1	7
Stoat #	3.28	1.79	1	7
Sturgeon	1.77	1.23	1	6
Swan	3.57	2.08	1	7
Swift #	2.24	2.01	1	7
Sword fish	2.31	2.04	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Tapir #	1.94	1.72	1	7
Tarantula #	2.54	1.65	1	6
Tench	2.02	1.98	1	7
Thrush	3.01	1.83	1	7
Tiger	6.20	0.99	4	7
Toad	2.81	1.89	1	7
Tortoise	3.17	1.84	1	7
Trout	2.32	2.01	1	7
Tuna	2.60	1.86	1	7
Turkey	4.06	1.79	2	7
Turtle	2.96	1.67	1	7
Tyrannosaurus	2.73	2.14	1	7
Vole	2.55	1.74	1	7
Vulture	2.60	1.96	1	7
Wallaby #	4.17	1.59	2	7
Walrus	4.02	1.66	2	7
Warthog	3.23	1.81	1	7
Wasp	2.25	2.16	1	7
Water buffalo #	3.05	2.08	1	7
Water rat	2.48	2.21	1	7
Weasel	3.41	1.81	1	7
Whale	3.40	2.06	1	7
White tiger	2.86	2.26	1	7
Wild boar	3.33	1.80	1	7
Wild cat	3.62	1.88	1	7
Wild dog ~	2.81	2.18	1	7
Wildebeest #	3.76	1.79	1	7
Wolf	5.08	1.56	2	7
Wombat	2.65	1.79	1	7
Woodcock	1.92	1.79	1	6
Woodlouse	1.62	1.54	1	6
Woodpecker	2.88	1.76	1	7
Worm	2.06	2.31	1	7
Wren	2.80	1.77	1	7
Yak #	2.78	2.12	1	7
Yellow tit	1.94	2.22	1	7
Yellowhammer #	1.81	1.65	1	6
Yorkshire terrier	5.89	1.09	3	7
Zebra	6.20	1.00	3	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.3b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to the 31-40 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	1.47	1.88	1	7
Almond	1.73	1.80	1	7
Apple	5.25	1.41	1	7
Apricot	5.61	1.10	4	7
Aubergine #	1.73	2.11	1	7
Avocado #	3.56	1.85	1	7
Banana	6.94	0.24	6	7
Berries	3.26	2.16	1	7
Bilberry #	1.71	2.30	1	7
Blackberry	4.78	1.64	3	7
Blackcurrant	4.19	1.70	1	7
Blueberry	4.31	1.81	2	7
Bramble #	2.78	1.99	1	7
Butternut squash	1.89	1.30	1	4
Cantaloupe melon	3.35	3.58	1	16
Cherry	4.81	1.55	1	7
Chestnut #	1.63	1.70	1	7
Citron	1.41	2.47	1	7
Clementine ~	4.30	1.69	1	7
Coconut	2.61	2.31	1	7
Cox apple # *	5.19	1.54	2	7
Crab apple	3.48	1.94	1	7
Cranberry	3.04	1.79	1	7
Cucumber #	2.30	2.22	1	7
Currant #	2.46	2.10	1	7
Damson #	2.47	1.56	1	7
Date	3.02	1.83	1	7
Dewberry #	1.57	1.95	1	7
Elderberry #	3.22	1.96	1	7
Fig #	2.79	1.76	1	7
Gala apple *	3.57	2.02	1	7
Galia melon # ~	3.18	2.20	1	7
Gooseberry #	3.89	1.93	1	7
Granny smith	5.76	1.19	3	7
Grapefruit	5.41	1.35	2	7

Fruit	Mean	SD	Range min	Range max
Grape	5.75	1.33	2	7
Green melon	3.92	1.73	2	7
Guava	2.04	1.76	1	6
Haw	1.39	1.87	1	7
Hazelnuts	1.73	1.80	1	7
Honeydew melon # ~	2.97	2.19	1	7
Horse chestnut	1.50	2.29	1	7
Jackfruit	1.31	1.58	1	5
Jaffa #	3.03	2.00	1	7
Kiwi	5.43	1.18	3	7
kumquat	1.89	1.73	1	7
Lemon	5.56	1.47	3	7
Lime	4.95	1.54	2	7
Loganberry #	1.89	1.91	1	6
Lychee # ~	2.50	1.90	1	6
Mandarin # ~	5.01	1.55	3	7
Mango	5.14	1.41	2	7
Melon	5.59	1.43	3	7
Nashi	1.07	0.35	1	2
Nectarine	5.57	1.10	3	7
Nuts # ~	1.45	1.86	1	7
Olive #	2.37	1.59	1	7
Orange	5.21	1.42	1	7
Papaya	2.53	2.08	1	7
Passion fruit	3.42	2.09	1	7
Paw paw #	2.29	2.09	1	7
Peach ~	5.96	1.06	3	7
Pear	6.21	0.98	4	7
Peppers	1.73	2.42	1	7
Persimmons	1.57	2.25	1	7
Pineapple	6.49	0.62	5	7
Plantain #	1.80	1.06	1	4
Plum	5.33	1.44	3	7
Pomelo	1.28	2.13	1	7
Pomegranate ~	3.04	1.97	1	7
Prune #	3.28	1.54	2	7
Quince	1.85	1.93	1	7
Raisin #	3.75	1.82	2	7
Raspberry	5.57	1.23	3	7

Fruit	Mean	SD	Range min	max
Red currant #	2.83	1.97	1	7
Redberry	2.56	1.87	1	7
Red grape	4.64	1.70	2	7
Rhubarb #	4.48	1.53	2	7
Rosehip # *	1.87	2.14	1	7
Satsuma ~	6.19	0.93	4	7
Sharon fruit ~	1.67	1.93	1	7
Sloe berry #	1.84	1.67	1	7
squash	1.88	1.77	1	7
Star fruit	2.67	1.71	1	7
Strawberry	6.61	0.59	5	7
Sultana	3.50	1.75	1	7
Tangerine	6.24	0.77	5	7
Tayberry	1.41	2.15	1	7
Tomato # *	3.43	2.30	1	7
Walnut #	1.57	1.87	1	7
Water melon	5.83	1.11	4	7
Whinberry	1.46	2.00	1	6
White currant #	1.73	1.71	1	6
White grape	3.90	1.99	1	7
Whortleberry	1.50	1.78	1	7

Table C.4a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to the 41-50 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	3.11	2.01	1	7
Adder	2.49	2.26	1	7
Albatross	2.18	1.77	1	7
Alligator	5.22	1.51	2	7
Alpaca #	2.68	2.02	1	7
Amoeba	1.32	2.14	1	7
Angel fish	2.11	1.96	1	7
Ant	2.27	2.59	1	7
Ant bear #	1.51	1.26	1	4
Anteater	3.82	1.65	2	7
Antelope #	5.55	1.19	4	7
Ape	5.98	1.14	3	7
Armadillo	3.90	1.82	2	7
Ass #	3.33	1.68	1	7
Baboon	5.22	1.51	2	7
Badger	4.83	1.61	2	7
Bald eagle	3.05	2.20	1	7
Bat	3.63	2.11	1	7
Bear	6.16	1.16	3	7
Beaver	5.18	1.22	4	7
Bee	2.65	2.55	1	7
Beetle	2.18	2.67	1	7
Bird #	3.17	2.41	1	7
Bison #	3.88	1.71	1	7
Blackbird	3.04	2.36	1	7
Blue bird	2.27	2.28	1	7
Blue tit	3.31	1.83	1	7
Boa constrictor	2.86	1.74	1	7
Boar #	4.54	1.29	3	7
Brontosaurus #	1.48	2.55	1	7
Brown bear	5.19	1.56	2	7
Buck #	3.02	1.49	1	7
Budgerigar	2.97	2.39	1	7
Buffalo	5.20	1.45	3	7
Bull	5.69	1.39	2	7

Animal	Mean	SD	Range min	Range max
Bullfinch # *	2.16	1.77	1	7
Bullock #	3.62	1.92	1	7
Butterfly	2.53	2.63	1	7
Buzzard	2.38	1.49	1	7
Calf ~	5.98	0.95	4	7
Camel #	6.04	1.14	4	7
Canary	2.40	2.30	1	7
Caribou #	2.95	1.90	1	7
Carp	2.07	1.88	1	7
Cat	6.64	0.80	4	7
Caterpillar	2.31	2.56	1	7
Cattle	5.09	1.70	3	7
Chaffinch	2.25	1.91	1	7
Chameleon	2.72	1.92	1	7
Cheetah	5.68	1.24	3	7
Chicken #	3.91	2.10	1	7
Chimpanzee	6.65	0.61	5	7
Chinchilla	3.77	1.70	1	7
Chipmunk	4.30	1.69	2	7
Clown fish	1.70	1.79	1	7
Cobra #	3.57	1.73	2	7
Cockatiel	2.40	2.05	1	7
Cocker spaniel ~	5.40	1.37	3	7
Cockerel	2.93	2.14	1	7
Cockroach	2.31	2.17	1	7
Cod	2.51	2.38	1	7
Condor #	2.07	1.64	1	7
Conger eel	2.08	2.02	1	7
Cougar #	4.16	1.59	2	7
Cow	6.81	0.53	5	7
Coyote	4.24	1.38	2	7
Cray fish	1.86	2.01	1	7
Crocodile	5.65	1.41	3	7
Crow	2.66	2.32	1	7
Cuckoo	3.07	2.12	1	7
Deer	6.22	0.84	4	7
Dingo #	3.22	1.86	1	7
Dog #	6.81	0.53	5	7
Dolphin	3.29	2.27	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	Range max
Donkey	6.55	0.74	5	7
Dormouse	3.68	1.64	2	7
Dove	3.03	2.17	1	7
Dragon	1.41	1.26	1	4
Dragonfly	2.47	2.23	1	7
Dromedary #	2.89	1.86	1	7
Duck	3.54	2.20	1	7
Duckbill platypus	2.46	1.66	1	6
Eagle	3.09	2.23	1	7
Earthworm	2.22	2.34	1	7
Earwig	2.30	2.28	1	7
Echidna	1.26	1.95	1	7
Elephant	2.22	2.24	1	7
Eel	5.69	1.39	2	7
Elk #	2.93	2.06	1	7
Emu	3.25	1.86	1	7
Ewe #	5.37	1.17	4	7
Ferret	3.54	1.90	1	7
Field mouse	3.57	1.70	1	7
Finch	2.45	2.07	1	7
Fish #	2.86	2.58	1	7
Flamingo	2.85	2.01	1	7
Flea	2.10	2.20	1	7
Fly	2.02	2.73	1	7
Fowl	2.52	2.28	1	7
Fox	6.43	0.85	4	7
Frog #	4.69	1.83	2	7
Gazelle #	4.12	1.55	2	7
Gecko	2.01	1.98	1	7
Gerbil	4.99	1.45	3	7
Giant panda	5.64	1.30	3	7
Gibbon	4.58	1.66	3	7
Giraffe	6.65	0.61	5	7
Gnu #	2.90	1.66	1	7
Goldcrest	6.50	0.84	4	7
Goat	1.66	0.82	1	3
Goldfish	2.73	2.30	1	7
Gorilla	2.98	2.23	1	7
Greyhound	6.55	0.74	5	7

Animal	Mean	SD	Range min	Range max
Grizzly bear	5.56	1.33	3	7
Groundhog	3.81	1.49	2	7
Guinea fowl	1.93	2.08	1	7
Guinea pig	4.93	1.45	2	7
Gull	2.84	2.23	1	7
Haddock	2.63	2.30	1	7
Hamster	6.14	0.95	4	7
Hare	4.55	1.52	3	7
Hart #	1.32	2.18	1	7
Hawk	2.91	2.21	1	7
Hedgehog	5.47	1.31	4	7
Heifer #	3.30	2.06	1	7
Hen	3.47	2.17	1	7
Heron	2.64	2.03	1	7
Herring	2.70	2.08	1	7
Hippopotamus	5.89	1.19	3	7
Hornet	1.65	2.22	1	7
Horse	6.81	0.53	5	7
Horsefly	1.92	2.02	1	7
Hyena	4.71	1.38	3	7
Ibex #	1.77	2.36	1	7
Iguana	2.94	1.83	1	7
Impala #	2.35	1.91	1	7
Insect	2.12	2.50	1	7
Invertebrate	1.40	2.17	1	7
Jack rabbit	2.82	1.90	1	7
Jackal #	4.41	1.62	2	7
Jackass #	3.01	1.72	1	7
Jackdaw #	2.18	1.61	1	7
Jaguar	4.09	1.74	1	7
Kangaroo	5.69	1.33	3	7
Kid #	2.89	2.05	1	7
Kitten	6.41	0.76	5	7
Kiwi	2.06	1.87	1	7
Koala	5.70	1.14	4	7
Koi carp	2.12	1.94	1	7
Komodo dragon	2.22	1.93	1	7
Lady bird	1.56	1.79	1	7
Lamb #	2.57	2.48	1	7

Animal	Mean	SD	Range min	max
Lemur	2.52	2.09	1	7
Leopard	5.45	1.41	3	7
Lion	4.47	1.86	1	7
Lizard	4.03	2.03	2	7
Llama #	4.04	1.55	2	7
Lobster	2.81	2.18	1	7
Long tailed tit	2.03	1.79	1	7
Lynx	2.66	1.99	1	7
Mackerel	2.25	1.98	1	7
Mammal	2.93	2.41	1	7
Manatee	2.71	1.39	1	5
Mandrill # *	1.78	1.99	1	7
Marmoset #	2.16	2.17	1	7
Marmot #	2.01	2.03	1	7
Marten #	1.49	1.38	1	5
Meerkat	4.73	1.24	3	7
Midge	1.76	2.21	1	7
Mink #	2.92	1.83	1	7
Manx	2.29	1.95	1	7
Mole	4.70	1.65	2	7
Mongoose #	2.90	1.88	1	7
Monkey #	6.65	0.61	5	7
Moose	5.04	1.28	3	7
Moth	2.31	2.56	1	7
Mouse ~	5.28	1.62	2	7
Mule	4.70	1.65	2	7
Musk ox #	1.78	2.04	1	7
Newt	2.44	2.39	1	7
Nightingale	2.05	2.50	1	7
Ocelot #	1.94	1.86	1	7
Octopus	2.98	2.36	1	7
Orang-utan	5.68	1.03	4	7
Oryx #	1.49	2.33	1	7
Ostrich	2.77	2.18	1	7
Otter #	5.61	1.05	4	7
Owl	3.21	2.16	1	7
Ox #	4.09	1.68	1	7
Panda	5.43	1.49	3	7
Panther	4.87	1.65	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Parakeet	2.55	1.86	1	7
Parrot	3.11	2.46	1	7
Partridge	3.12	1.88	1	7
Peacock	3.55	1.86	1	7
Peewit #	1.60	1.64	1	7
Pelican	3.05	1.82	1	7
Penguin ~	3.27	2.15	1	7
Perch	2.09	2.11	1	7
Pheasant	3.82	2.02	1	7
Pig	6.92	0.27	6	7
Pigeon	2.97	2.40	1	7
Piglet ~	5.25	1.44	3	7
Pike	2.55	2.13	1	7
Pine marten #	1.91	1.94	1	7
Piranha fish	2.43	1.99	1	7
Plaice	2.97	1.94	1	7
Platypus	2.90	1.96	1	7
Polar bear	6.26	0.94	4	7
Polar cat	2.82	1.94	1	7
Pony	6.65	0.61	5	7
Porcupine	3.28	1.91	1	7
Porpoise #	2.76	1.98	1	7
Poultry	3.13	2.13	1	7
Prairie dog	4.28	1.56	2	7
Puffin	3.03	1.95	1	7
Puma	3.19	1.98	1	7
Rabbit	6.81	0.53	5	7
Racoon	4.02	1.61	1	7
Ram #	5.13	1.34	4	7
Rat	5.89	1.33	3	7
Raven	2.76	2.09	1	7
Reindeer	4.66	1.81	2	7
Rhesus monkey #	2.80	2.23	1	7
Rhinoceros	5.83	1.14	4	7
Roach	1.90	1.72	1	7
Robin	2.99	2.33	1	7
Rodent	3.17	2.06	1	7
Roe dear #	3.27	2.34	1	7
Rook	2.00	1.94	1	7

Animal	Mean	SD	Range min	max
Rooster	3.38	2.03	1	7
Salamander	1.95	1.80	1	7
Salmon	2.67	2.28	1	7
Sardine	2.26	2.18	1	7
Sea lion	2.61	2.37	1	7
Seagull	2.90	2.58	1	7
Seahorse	2.13	2.28	1	7
Seal	3.85	1.94	1	7
Shark #	3.59	2.26	1	7
Sheep	6.33	0.94	4	7
Short tailed tit	1.85	1.88	1	7
Shrew	4.45	1.54	3	7
Shrimp	2.99	2.14	1	7
Siamese cat ~	3.79	2.09	1	7
Siberian tiger	3.87	1.86	1	7
Skate	2.06	1.96	1	7
Skunk	4.06	1.55	2	7
Skylark #	2.25	1.72	1	7
Sloth	2.69	1.98	1	7
Slug	1.79	2.16	1	7
Snail	2.80	2.41	1	7
Snake #	3.65	2.23	1	7
Sole	1.86	1.77	1	7
Sow #	3.21	2.24	1	7
Sparrow	3.04	2.41	1	7
Spider	3.03	2.48	1	7
Springbok #	2.45	2.03	1	7
Squid	2.14	2.24	1	7
Squirrel	6.26	1.09	3	7
Stag	4.28	1.70	1	7
Star fish	2.07	1.94	1	7
Starling	2.13	2.43	1	7
Stick insect	1.89	1.70	1	7
Stickleback	2.05	2.15	1	7
Stoat #	4.16	1.59	2	7
Sturgeon	2.02	1.67	1	7
Swan	3.23	2.40	1	7
Swift #	2.83	1.96	1	7
Sword fish	2.54	1.98	1	7

~ significant effect of age, **#** significant effect of gender, ***significant effect of education

Animal	Mean	SD	Range min	max
Tapir #	2.07	2.20	1	7
Tarantula #	3.16	1.89	1	7
Tench	1.83	1.90	1	7
Thrush	2.89	2.31	1	7
Tiger	6.64	0.80	4	7
Toad	3.47	1.95	1	7
Tortoise	3.62	2.22	1	7
Trout	2.70	2.35	1	7
Tuna	2.59	1.98	1	7
Turkey	3.00	2.38	1	7
Turtle	2.74	2.21	1	7
Tyrannosaurus	1.77	2.14	1	7
Vole	3.40	1.65	1	7
Vulture	2.94	1.83	1	7
Wallaby #	3.59	1.89	1	7
Walrus	4.06	1.82	1	7
Warthog	2.84	2.23	1	7
Wasp	2.55	2.50	1	7
Water buffalo #	3.82	1.88	1	7
Water rat	2.70	1.68	1	7
Weasel	4.26	1.66	2	7
Whale	4.56	1.73	1	7
White tiger	3.23	2.01	1	7
Wild boar	5.14	1.28	4	7
Wild cat	3.23	1.89	1	7
Wild dog ~	3.50	1.91	1	7
Wildebeest #	3.90	1.86	1	7
Wolf	6.29	0.85	4	7
Wombat	3.97	1.70	2	7
Woodcock	1.94	2.04	1	7
Woodlouse	2.39	2.39	1	7
Woodpecker	2.68	2.11	1	7
Worm	2.35	2.62	1	7
Wren	2.37	2.06	1	7
Yak #	4.66	1.49	3	7
Yellow tit	2.04	1.82	1	7
Yellowhammer #	1.86	1.70	1	7
Yorkshire terrier	5.83	1.14	4	7
Zebra	6.33	0.94	4	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.4b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to the 41-50 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	1.47	1.78	1	6
Almond	2.02	2.77	1	7
Apple	7.00	0.00	7	7
Apricot	6.24	0.84	5	7
Aubergine #	2.40	2.19	1	7
Avocado #	2.66	2.25	1	7
Banana	7.00	0.00	7	7
Berries	6.17	1.01	4	7
Bilberry #	1.72	2.44	1	7
Blackberry	6.22	0.93	5	7
Blackcurrant	6.11	0.99	4	7
Blueberry	5.89	1.19	3	7
Bramble #	2.92	2.06	1	7
Butternut squash	1.86	1.66	1	6
Cantaloupe melon	3.88	1.82	1	7
Cherry	6.13	1.08	4	7
Chestnut #	2.15	2.49	1	7
Citron	1.82	2.39	1	7
Clementine ~	4.31	1.82	1	7
Coconut	2.83	2.13	1	7
Cox apple # *	5.62	1.36	3	7
Crab apple	3.59	2.01	1	7
Cranberry	5.06	1.45	2	7
Cucumber #	1.88	2.77	1	7
Currant #	5.28	1.49	3	7
Damson #	3.04	2.06	1	7
Date	3.68	1.92	1	7
Dewberry #	1.87	2.25	1	7
Elderberry #	3.30	1.99	1	7
Fig #	3.42	1.83	1	7
Gala apple *	3.90	2.03	1	7
Galia melon # ~	3.70	2.02	1	7
Gooseberry #	5.24	1.34	3	7
Granny smith	6.62	0.67	5	7
Grapefruit	6.58	0.63	5	7

Fruit	Mean	SD	Range min	Range max
Grape	6.92	0.27	6	7
Green melon	2.93	2.14	1	7
Guava	1.86	2.20	1	7
Haw	1.13	0.92	1	4
Hazelnuts	2.15	2.30	1	7
Honeydew melon # ~	5.50	1.29	3	7
Horse chestnut	2.24	2.20	1	7
Jackfruit	1.25	1.48	1	6
Jaffa #	4.00	1.96	2	7
Kiwi	5.13	1.34	4	7
kumquat	1.85	2.16	1	7
Lemon	6.92	0.27	6	7
Lime	6.31	0.85	5	7
Loganberry #	3.02	2.03	1	7
Lychee # ~	2.46	1.87	1	7
Mandarin # ~	5.35	1.47	2	7
Mango	4.85	1.56	2	7
Melon	6.50	0.65	5	7
Nashi	1.08	0.95	1	4
Nectarine	4.22	1.74	1	7
Nuts # ~	2.24	2.66	1	7
Olive #	3.96	2.04	2	7
Orange	7.00	0.00	7	7
Papaya	2.38	2.12	1	7
Passion fruit	5.21	1.22	3	7
Paw paw #	1.94	2.47	1	7
Peach ~	6.50	0.84	4	7
Pear	6.84	0.36	6	7
Peppers	2.45	2.27	1	7
Persimmons	1.25	1.04	1	4
Pineapple	6.26	1.09	3	7
Plantain #	1.49	1.72	1	7
Plum	6.33	0.94	4	7
Pomelo	1.15	1.38	1	5
Pomegranate ~	4.54	1.64	2	7
Prune #	4.80	1.59	3	7
Quince	1.63	1.83	1	7
Raisin #	4.57	1.73	2	7
Raspberry	6.76	0.43	6	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	max
Red currant #	4.15	1.77	2	7
Redberry	2.69	2.29	1	7
Red grape	4.46	1.91	2	7
Rhubarb #	5.62	1.21	3	7
Rosehip # *	1.72	2.16	1	7
Satsuma ~	5.38	1.54	2	7
Sharon fruit ~	1.99	2.02	1	7
Sloe berry #	2.57	1.48	1	6
squash	2.10	1.89	1	7
Star fruit	2.03	2.10	1	7
Strawberry	6.39	1.07	3	7
Sultana	3.59	2.07	2	7
Tangerine	6.45	0.85	5	7
Tayberry	1.20	1.76	1	7
Tomato # *	5.57	1.49	2	7
Walnut #	2.08	2.58	1	7
Water melon	5.90	1.00	4	7
Whinberry	1.39	2.09	1	7
White currant #	1.74	2.30	1	7
White grape	2.77	2.51	1	7
Whortleberry	1.14	1.51	1	6

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.5a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to the 51-60 age –category.

Animal	**Mean**	**SD**	**Range** min	max
Aardvark	1.67	2.46	1	7
Adder	3.60	1.75	1	7
Albatross	2.29	2.09	1	7
Alligator	4.67	1.71	1	7
Alpaca #	1.98	2.62	1	7
Amoeba	1.51	2.32	1	7
Angel fish	1.74	2.01	1	7
Ant	2.49	2.33	1	7
Ant bear #	1.83	2.47	1	7
Anteater	3.25	2.12	1	7
Antelope #	4.01	1.90	1	7
Ape	6.34	0.92	4	7
Armadillo	2.71	2.24	1	7
Ass #	3.32	1.94	1	7
Baboon	4.08	1.91	1	7
Badger	5.53	1.35	3	7
Bald eagle	1.96	2.08	1	6
Bat	4.20	2.09	2	7
Bear	6.64	0.67	5	7
Beaver	3.82	2.07	1	7
Bee	2.63	2.41	1	7
Beetle	2.28	2.34	1	7
Bird #	3.00	2.38	1	7
Bison #	3.36	2.20	1	7
Blackbird	3.39	2.11	1	7
Blue bird	2.36	1.93	1	7
Blue tit	2.47	2.15	1	7
Boa constrictor	2.76	2.14	1	7
Boar #	3.97	1.82	1	7
Brontosaurus #	2.28	2.45	1	7
Brown bear	4.48	1.77	1	7
Buck #	1.97	2.52	1	7
Budgerigar	2.82	2.35	1	7
Buffalo	4.69	1.64	2	7
Bull	5.71	1.37	3	7

Animal	Mean	SD	Range min	Range max
Bullfinch # *	1.67	1.88	1	6
Bullock #	4.87	1.42	2	7
Butterfly	2.84	2.54	1	7
Buzzard	2.06	2.23	1	7
Calf ~	5.50	1.17	3	7
Camel #	5.89	1.33	3	7
Canary	2.63	2.14	1	7
Caribou #	2.64	2.45	1	7
Carp	2.25	2.06	1	7
Cat	6.87	0.32	6	7
Caterpillar	2.92	2.22	1	7
Cattle	6.34	0.63	5	7
Chaffinch	1.94	1.86	1	6
Chameleon	3.44	1.74	1	7
Cheetah	5.77	1.23	3	7
Chicken #	3.73	2.11	1	7
Chimpanzee	6.44	0.72	5	7
Chinchilla	3.21	2.21	1	7
Chipmunk	3.01	2.34	1	7
Clown fish	1.63	2.36	1	7
Cobra #	2.77	2.15	1	7
Cockatiel	2.36	2.08	1	7
Cocker spaniel ~	5.12	1.33	2	7
Cockerel	3.05	2.23	1	7
Cockroach	2.12	2.43	1	7
Cod	2.85	2.42	1	7
Condor #	2.11	2.12	1	6
Conger eel	1.83	1.86	1	6
Cougar #	2.52	2.55	1	7
Cow	6.75	0.43	6	7
Coyote	2.94	2.31	1	7
Cray fish	2.01	1.84	1	6
Crocodile	6.13	1.03	4	7
Crow	2.77	1.95	1	7
Cuckoo	3.13	2.06	1	7
Deer	5.82	1.29	2	7
Dingo #	3.37	2.04	1	7
Dog #	6.79	0.51	5	7
Dolphin	6.40	0.79	5	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Donkey	6.06	1.08	3	7
Dormouse	3.51	2.13	1	7
Dove	3.16	2.17	1	7
Dragon	2.12	2.46	1	7
Dragonfly	2.28	2.22	1	7
Dromedary #	2.12	2.32	1	7
Duck	3.61	2.09	1	7
Duckbill platypus	1.79	1.98	1	7
Eagle	3.50	2.05	1	7
Earthworm	3.05	2.20	1	7
Earwig	2.26	2.12	1	7
Echidna	1.27	1.28	1	5
Elephant	2.51	2.05	1	7
Eel	6.72	0.71	4	7
Elk #	2.77	2.38	1	7
Emu	2.42	2.21	1	7
Ewe #	3.55	2.06	1	7
Ferret	4.47	1.65	1	7
Field mouse	3.75	1.98	1	7
Finch	2.04	2.44	1	7
Fish #	3.01	2.43	1	7
Flamingo	3.37	1.93	1	7
Flea	2.14	2.30	1	7
Fly	2.40	2.70	1	7
Fowl	2.63	2.14	1	7
Fox	6.00	1.19	3	7
Frog #	3.13	2.30	1	7
Gazelle #	4.04	1.84	1	7
Gecko	1.90	2.13	1	7
Gerbil	5.76	1.00	4	7
Giant panda	4.41	1.82	1	7
Gibbon	3.12	2.04	1	7
Giraffe	6.00	1.18	4	7
Gnu #	2.18	2.48	1	7
Goldcrest	6.11	1.08	4	7
Goat	1.52	1.69	1	6
Goldfish	2.84	2.38	1	7
Gorilla	3.50	1.86	1	7
Greyhound	5.75	1.40	3	7

Animal	Mean	SD	Range min	max
Grizzly bear	5.28	1.55	2	7
Groundhog	2.37	2.35	1	7
Guinea fowl	1.91	1.82	1	7
Guinea pig	5.66	1.35	3	7
Gull	2.70	2.03	1	7
Haddock	2.64	2.43	1	7
Hamster	5.36	1.51	2	7
Hare	4.20	1.85	1	7
Hart #	2.27	2.33	1	7
Hawk	2.70	2.27	1	7
Hedgehog	6.38	0.79	4	7
Heifer #	4.02	1.81	2	7
Hen	3.03	2.16	1	7
Heron	2.34	2.23	1	7
Herring	2.26	1.89	1	6
Hippopotamus	6.12	1.15	3	7
Hornet	1.94	1.76	1	7
Horse	6.41	0.86	4	7
Horsefly	1.80	1.85	1	7
Hyena	4.24	1.76	1	7
Ibex #	1.73	2.33	1	7
Iguana	3.02	1.90	1	7
Impala #	2.40	2.34	1	7
Insect	2.10	2.73	1	7
Invertebrate	1.86	2.09	1	7
Jack rabbit	2.00	2.56	1	7
Jackal #	3.31	2.12	1	7
Jackass #	2.49	2.27	1	7
Jackdaw #	2.11	1.80	1	6
Jaguar	4.58	1.87	2	7
Kangaroo	6.11	0.96	4	7
Kid #	3.49	2.12	1	7
Kitten	6.41	0.86	4	7
Kiwi	2.07	1.90	1	7
Koala	5.18	1.45	2	7
Koi carp	2.39	1.82	1	6
Komodo dragon	2.05	2.38	1	7
Lady bird	1.36	1.25	1	5
Lamb #	2.83	2.38	1	7

Animal	Mean	SD	Range min	max
Lemur	2.32	2.46	1	7
Leopard	5.87	1.26	3	7
Lion	6.75	0.43	6	7
Lizard	3.26	2.19	1	7
Llama #	3.61	1.98	1	7
Lobster	2.63	2.33	1	7
Long tailed tit	2.17	1.90	1	6
Lynx	2.76	2.28	1	7
Mackerel	1.86	2.13	1	7
Mammal	4.57	1.76	1	7
Manatee	2.12	2.38	1	7
Mandrill # *	1.60	2.31	1	7
Marmoset #	2.28	2.19	1	7
Marmot #	1.52	2.56	1	7
Marten #	1.91	2.06	1	7
Meerkat	3.96	1.98	1	7
Midge	2.26	2.12	1	7
Mink #	3.34	2.01	1	7
Manx	2.42	2.46	1	7
Mole	4.22	1.92	1	7
Mongoose #	2.26	2.64	1	8
Monkey #	6.63	0.49	6	7
Moose	2.87	2.33	1	7
Moth	2.66	2.45	1	7
Mouse ~	5.54	1.47	2	7
Mule	3.87	1.96	1	7
Musk ox #	1.73	2.55	1	7
Newt	2.40	2.09	1	7
Nightingale	2.28	1.94	1	7
Ocelot #	1.97	2.52	1	7
Octopus	3.21	1.91	1	7
Orang-utan	5.00	1.65	2	7
Oryx #	1.48	2.13	1	7
Ostrich	3.21	2.05	1	7
Otter #	5.20	1.58	2	7
Owl	3.65	1.99	1	7
Ox #	3.60	2.22	1	7
Panda	5.84	1.31	3	7
Panther	5.15	1.62	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Parakeet	2.27	2.09	1	7
Parrot	3.50	2.04	1	7
Partridge	2.58	1.90	1	7
Peacock	2.96	2.07	1	7
Peewit #	1.51	1.93	1	6
Pelican	2.59	2.14	1	7
Penguin ~	4.14	1.85	1	7
Perch	1.86	1.79	1	7
Pheasant	3.16	2.03	1	7
Pig	6.49	0.78	4	7
Pigeon	3.35	2.06	1	7
Piglet ~	5.90	1.08	4	7
Pike	2.25	1.81	1	7
Pine marten #	1.87	2.36	1	7
Piranha fish	1.99	2.20	1	7
Plaice	2.55	2.18	1	7
Platypus	2.21	2.25	1	7
Polar bear	6.47	0.70	5	7
Polar cat	2.13	2.44	1	7
Pony	6.14	0.73	5	7
Porcupine	3.77	1.72	1	7
Porpoise #	2.34	2.26	1	7
Poultry	3.06	2.28	1	7
Prairie dog	2.62	2.19	1	7
Puffin	2.47	1.99	1	7
Puma	5.06	1.50	2	7
Rabbit	6.75	0.43	6	7
Racoon	3.10	2.23	1	7
Ram #	4.87	1.64	2	7
Rat	4.77	1.64	1	7
Raven	2.90	1.81	1	7
Reindeer	5.74	1.27	2	7
Rhesus monkey #	3.41	2.06	1	7
Rhinoceros	4.37	1.83	1	7
Roach	1.58	1.96	1	7
Robin	3.31	2.32	1	7
Rodent	4.66	1.74	2	7
Roe dear #	3.90	1.87	1	7
Rook	2.18	2.01	1	6

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Rooster	2.31	2.35	1	7
Salamander	1.61	1.97	1	6
Salmon	2.72	2.50	1	7
Sardine	2.08	2.03	1	7
Sea lion	4.05	1.81	1	7
Seagull	3.13	2.20	1	7
Seahorse	2.28	2.29	1	7
Seal	6.23	0.92	4	7
Shark #	3.73	2.01	1	7
Sheep	6.59	0.69	5	7
Short tailed tit	2.19	2.18	1	7
Shrew	3.23	2.11	1	7
Shrimp	2.84	2.13	1	7
Siamese cat ~	5.43	1.34	3	7
Siberian tiger	3.00	2.39	1	7
Skate	1.85	1.82	1	7
Skunk	2.68	2.40	1	7
Skylark #	2.72	1.64	1	6
Sloth	2.59	2.38	1	7
Slug	2.32	2.51	1	7
Snail	2.49	2.54	1	7
Snake #	3.35	2.34	1	7
Sole	1.99	1.97	1	7
Sow #	4.72	1.70	2	7
Sparrow	3.04	2.39	1	7
Spider	2.85	2.42	1	7
Springbok #	2.49	2.42	1	7
Squid	2.56	1.84	1	7
Squirrel	6.19	0.93	4	7
Stag	3.50	2.10	1	7
Star fish	2.39	2.04	1	7
Starling	2.82	2.31	1	7
Stick insect	1.77	1.75	1	7
Stickleback	1.90	1.95	1	6
Stoat #	2.70	2.29	1	7
Sturgeon	1.77	1.65	1	7
Swan	3.49	2.03	1	7
Swift #	2.65	1.94	1	7
Sword fish	2.12	2.11	1	7

Animal	Mean	SD	Range min	max
Tapir #	2.14	2.18	1	7
Tarantula #	2.06	2.33	1	7
Tench	1.58	2.15	1	7
Thrush	2.70	2.35	1	7
Tiger	6.67	0.57	5	7
Toad	3.29	2.05	1	7
Tortoise	4.41	1.78	1	7
Trout	2.13	2.47	1	7
Tuna	2.46	2.09	1	7
Turkey	3.01	2.31	1	7
Turtle	3.78	1.84	1	7
Tyrannosaurus	2.50	2.32	1	7
Vole	3.86	1.98	2	7
Vulture	2.71	2.01	1	7
Wallaby #	3.44	2.14	1	7
Walrus	3.25	2.13	1	7
Warthog	2.18	2.37	1	7
Wasp	2.43	2.59	1	7
Water buffalo #	3.09	2.34	1	7
Water rat	3.07	2.15	1	7
Weasel	3.91	2.08	1	7
Whale	4.80	1.64	1	7
White tiger	3.07	2.21	1	7
Wild boar	3.66	2.01	1	7
Wild cat	2.68	2.38	1	7
Wild dog ~	3.81	2.08	1	7
Wildebeest #	2.83	2.33	1	7
Wolf	5.46	1.45	2	7
Wombat	3.06	2.09	1	7
Woodcock	1.76	2.06	1	7
Woodlouse	2.31	2.32	1	7
Woodpecker	2.45	2.14	1	7
Worm	2.60	2.50	1	7
Wren	2.52	2.11	1	7
Yak #	2.15	2.44	1	7
Yellow tit	1.68	2.01	1	6
Yellowhammer #	1.96	1.93	1	6
Yorkshire terrier	4.86	1.39	1	7
Zebra	5.75	1.38	3	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.5b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to the 51-60 age –category.

Fruit	**Mean**	**SD**	**Range** min	max
Acorn	1.91	2.10	1	7
Almond	3.29	1.88	1	7
Apple	6.64	0.67	5	7
Apricot	5.31	1.37	3	7
Aubergine #	2.39	1.82	1	7
Avocado #	3.85	1.80	1	7
Banana	6.79	0.51	5	7
Berries	5.58	1.48	2	7
Bilberry #	2.82	2.18	1	7
Blackberry	5.15	1.52	2	7
Blackcurrant	5.94	1.11	3	7
Blueberry	4.24	1.75	1	7
Bramble #	4.34	1.72	1	7
Butternut squash	1.98	2.05	1	7
Cantaloupe melon	4.68	1.69	2	7
Cherry	6.23	1.04	3	7
Chestnut #	2.89	1.92	1	7
Citron	2.38	2.15	1	7
Clementine ~	4.46	1.71	1	7
Coconut	3.73	2.01	1	7
Cox apple # *	5.80	1.18	3	7
Crab apple	3.54	1.82	1	7
Cranberry	3.89	1.93	1	7
Cucumber #	2.61	2.49	1	7
Currant #	5.15	1.64	2	7
Damson #	4.05	1.94	2	7
Date	4.86	1.58	3	7
Dewberry #	2.04	2.19	1	7
Elderberry #	3.88	1.82	1	7
Fig #	4.86	1.49	3	7
Gala apple *	3.94	1.96	1	7
Galia melon # ~	4.40	1.90	2	7
Gooseberry #	5.26	1.49	2	7
Granny smith	6.02	1.01	4	7
Grapefruit	6.28	0.92	4	7

Fruit	Mean	SD	Range	
			min	max
Grape	6.67	0.57	5	7
Green melon	2.59	2.32	1	7
Guava	2.35	2.29	1	7
Haw	1.70	2.14	1	7
Hazelnuts	3.06	2.03	1	7
Honeydew melon # ~	5.31	1.44	3	7
Horse chestnut	2.49	2.05	1	7
Jackfruit	1.50	1.91	1	7
Jaffa #	5.47	1.33	2	7
Kiwi	5.65	1.28	3	7
kumquat	1.92	2.50	1	7
Lemon	4.86	1.67	1	7
Lime	4.22	1.59	1	7
Loganberry #	2.65	1.97	1	7
Lychee # ~	4.28	1.62	2	7
Mandarin # ~	5.74	1.21	3	7
Mango	5.12	1.59	2	7
Melon	6.36	0.86	4	7
Nashi	1.13	1.20	1	5
Nectarine	5.74	1.19	4	7
Nuts # ~	3.83	2.03	1	7
Olive #	2.69	2.28	1	7
Orange	6.79	0.51	5	7
Papaya	3.68	2.03	2	7
Passion fruit	4.88	1.50	2	7
Paw paw #	2.62	2.11	1	7
Peach ~	6.55	0.77	4	7
Pear	6.67	0.57	5	7
Peppers	2.70	2.23	1	7
Persimmons	1.72	2.12	1	7
Pineapple	6.41	0.86	4	7
Plantain #	1.74	2.36	1	7
Plum	6.49	0.78	4	7
Pomelo	1.43	2.26	1	7
Pomegranate ~	5.20	1.29	3	7
Prune #	4.19	1.82	1	7
Quince	1.95	2.31	1	7
Raisin #	5.40	1.38	3	7
Raspberry	5.60	1.34	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	Range max
Red currant #	4.23	1.64	2	7
Redberry	2.91	2.18	1	7
Red grape	5.63	1.43	3	7
Rhubarb #	4.11	1.92	1	7
Rosehip # *	2.67	1.81	1	7
Satsuma ~	5.91	1.17	3	7
Sharon fruit ~	1.97	2.68	1	7
Sloe berry #	2.04	2.16	1	7
squash	2.39	2.21	1	7
Star fruit	3.35	1.85	1	7
Strawberry	6.75	0.43	6	7
Sultana	4.93	1.58	2	7
Tangerine	5.47	1.34	3	7
Tayberry	2.17	2.09	1	7
Tomato # *	4.45	1.91	1	7
Walnut #	3.02	1.83	1	7
Water melon	5.52	1.39	3	7
Whinberry	1.61	2.31	1	7
White currant #	1.92	2.16	1	7
White grape	3.73	2.10	1	7
Whortleberry	1.68	2.24	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.6a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to the 61-70 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark	2.91	2.04	1	7
Adder	2.27	1.95	1	7
Albatross	2.87	1.71	1	7
Alligator	3.40	2.19	1	7
Alpaca #	3.37	2.04	1	7
Amoeba	1.03	0.25	1	2
Angel fish	1.47	1.63	1	7
Ant	1.26	1.93	1	7
Ant bear #	2.71	2.35	1	7
Anteater	3.33	2.00	1	7
Antelope #	5.99	1.10	3	7
Ape	6.43	0.77	5	7
Armadillo	3.17	2.09	1	7
Ass #	4.43	1.71	1	7
Baboon	4.74	1.70	3	7
Badger	4.52	1.54	1	7
Bald eagle	2.93	2.00	1	7
Bat	2.45	2.12	1	7
Bear	5.57	1.33	2	7
Beaver	4.08	1.87	1	7
Bee	1.67	1.92	1	7
Beetle	1.39	1.99	1	7
Bird #	2.45	2.14	1	7
Bison #	4.05	1.98	1	7
Blackbird	2.66	1.83	1	7
Blue bird	2.44	1.93	1	7
Blue tit	2.46	2.09	1	7
Boa constrictor	2.27	2.04	1	7
Boar #	5.21	1.42	3	7
Brontosaurus #	2.69	2.41	1	7
Brown bear	5.82	1.15	3	7
Buck #	3.40	2.05	1	7
Budgerigar	2.68	2.18	1	7
Buffalo	4.85	1.54	1	7
Bull	6.39	0.84	4	7

Animal	Mean	SD	Range min	max
Bullfinch # *	2.56	1.89	1	7
Bullock #	5.64	1.30	2	7
Butterfly	1.52	2.38	1	7
Buzzard	2.67	2.05	1	7
Calf ~	4.72	1.59	1	7
Camel #	6.27	0.76	5	7
Canary	2.50	2.08	1	7
Caribou #	4.21	1.77	1	7
Carp	1.68	1.61	1	7
Cat	5.88	1.26	2	7
Caterpillar	1.28	1.94	1	7
Cattle	6.57	0.60	5	7
Chaffinch	2.14	2.03	1	7
Chameleon	2.34	1.94	1	7
Cheetah	6.25	0.83	5	7
Chicken #	3.22	1.95	1	7
Chimpanzee	5.27	1.49	2	7
Chinchilla	3.31	2.09	1	7
Chipmunk	3.59	2.06	1	7
Clown fish	1.43	1.35	1	6
Cobra #	2.40	1.83	1	7
Cockatiel	2.60	1.98	1	7
Cocker spaniel ~	5.70	1.20	3	7
Cockerel	3.19	1.90	1	7
Cockroach	1.30	1.87	1	7
Cod	1.93	2.11	1	7
Condor #	2.83	1.80	1	7
Conger eel	2.02	2.01	1	7
Cougar #	5.10	1.53	2	7
Cow	6.76	0.42	6	7
Coyote	3.33	2.16	1	7
Cray fish	1.93	1.67	1	7
Crocodile	3.53	2.02	1	7
Crow	2.53	1.95	1	7
Cuckoo	2.66	2.02	1	7
Deer	5.96	1.01	4	7
Dingo #	3.86	1.89	1	7
Dog #	4.96	1.60	1	7
Dolphin	3.76	1.82	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Donkey	5.87	1.12	3	7
Dormouse	3.57	2.08	1	7
Dove	2.63	1.90	1	7
Dragon	2.13	2.56	1	7
Dragonfly	1.32	1.57	1	7
Dromedary #	3.27	2.38	1	7
Duck	3.73	1.77	1	7
Duckbill platypus	1.47	1.96	1	7
Eagle	2.91	1.97	1	7
Earthworm	1.35	2.13	1	7
Earwig	1.38	2.02	1	7
Echidna	1.41	1.41	1	5
Elephant	1.65	1.98	1	7
Eel	6.82	0.37	6	7
Elk #	4.45	1.64	1	7
Emu	3.99	1.90	2	7
Ewe #	3.55	2.16	1	7
Ferret	3.63	1.91	1	7
Field mouse	3.47	2.04	1	7
Finch	2.57	1.87	1	7
Fish #	1.99	1.87	1	7
Flamingo	2.98	1.85	1	7
Flea	1.20	1.46	1	7
Fly	1.31	1.99	1	7
Fowl	2.58	2.31	1	7
Fox	4.49	1.67	1	7
Frog #	2.31	1.95	1	7
Gazelle #	5.60	1.03	4	7
Gecko	1.94	2.15	1	7
Gerbil	3.16	2.17	1	7
Giant panda	6.01	1.05	3	7
Gibbon	4.52	1.89	2	7
Giraffe	5.51	1.45	2	7
Gnu #	2.19	2.62	1	7
Goldcrest	6.11	0.93	4	7
Goat	2.08	1.93	1	7
Goldfish	1.79	2.17	1	7
Gorilla	2.78	2.03	1	7
Greyhound	6.17	1.02	3	7

Animal	Mean	SD	Range min	max
Grizzly bear	5.55	1.41	3	7
Groundhog	2.81	2.37	1	7
Guinea fowl	2.62	1.98	1	7
Guinea pig	4.30	1.87	2	7
Gull	2.31	2.06	1	7
Haddock	1.97	2.13	1	7
Hamster	3.49	2.22	1	7
Hare	5.37	1.25	3	7
Hart #	2.16	2.56	1	7
Hawk	2.64	2.04	1	7
Hedgehog	3.56	2.06	1	7
Heifer #	4.92	1.54	1	7
Hen	3.31	2.14	1	7
Heron	2.94	1.84	1	7
Herring	2.15	1.86	1	7
Hippopotamus	5.62	1.35	3	7
Hornet	1.36	1.67	1	7
Horse	5.28	1.38	1	7
Horsefly	1.29	1.85	1	7
Hyena	3.92	1.97	1	7
Ibex #	2.84	2.27	1	7
Iguana	2.79	2.07	1	7
Impala #	3.32	2.24	1	7
Insect	1.40	1.84	1	7
Invertebrate	1.18	1.15	1	5
Jack rabbit	3.65	1.97	1	7
Jackal #	3.40	2.16	1	7
Jackass #	3.77	1.80	1	7
Jackdaw #	2.76	1.85	1	7
Jaguar	5.26	1.52	2	7
Kangaroo	5.49	1.33	3	7
Kid #	3.12	2.14	1	7
Kitten	4.88	1.61	2	7
Kiwi	1.89	1.97	1	7
Koala	3.94	1.87	1	7
Koi carp	1.80	1.95	1	7
Komodo dragon	2.16	2.18	1	7
Lady bird	1.17	1.46	1	6
Lamb #	1.50	2.06	1	7

Animal	Mean	SD	Range min	max
Lemur	3.47	2.09	1	7
Leopard	6.11	0.93	4	7
Lion	6.71	0.45	6	7
Lizard	2.46	1.92	1	7
Llama #	4.94	1.64	2	7
Lobster	2.48	1.80	1	7
Long tailed tit	2.31	1.68	1	7
Lynx	3.61	1.97	1	7
Mackerel	2.17	1.93	1	7
Mammal	5.38	1.43	2	7
Manatee	1.44	1.83	1	5
Mandrill # *	2.09	2.50	1	7
Marmoset #	3.03	2.21	1	7
Marmot #	2.80	2.21	1	7
Marten #	1.88	2.40	1	7
Meerkat	2.93	2.30	1	7
Midge	1.11	1.39	1	7
Mink #	3.98	1.71	2	7
Manx	3.11	2.04	1	7
Mole	3.82	1.87	1	7
Mongoose #	3.31	2.17	1	7
Monkey #	4.95	1.63	2	7
Moose	6.17	0.89	4	7
Moth	1.49	2.03	1	7
Mouse ~	4.23	1.95	1	7
Mule	5.49	1.33	3	7
Musk ox #	3.11	2.32	1	7
Newt	1.75	2.02	1	7
Nightingale	2.76	1.89	1	7
Ocelot #	2.16	2.62	1	7
Octopus	2.23	1.85	1	7
Orang-utan	5.68	1.33	3	7
Oryx #	2.31	2.25	1	7
Ostrich	3.78	1.67	1	7
Otter #	4.73	1.64	2	7
Owl	3.28	1.72	1	7
Ox #	5.44	1.45	3	7
Panda	5.52	1.25	2	7
Panther	5.81	1.15	3	7

Animal	Mean	SD	Range min	max
Parakeet	2.85	1.87	1	7
Parrot	2.63	2.09	1	7
Partridge	2.67	1.91	1	7
Peacock	2.73	2.25	1	7
Peewit #	2.03	1.72	1	7
Pelican	2.93	1.97	1	7
Penguin ~	4.88	1.58	2	7
Perch	1.87	1.47	1	7
Pheasant	2.77	1.86	1	7
Pig	6.25	0.83	5	7
Pigeon	2.53	2.00	1	7
Piglet ~	5.45	1.31	2	7
Pike	2.00	1.61	1	7
Pine marten #	2.20	2.37	1	7
Piranha fish	1.78	1.80	1	7
Plaice	1.74	2.12	1	7
Platypus	2.43	2.04	1	7
Polar bear	6.13	1.00	4	7
Polar cat	2.69	2.42	1	7
Pony	5.83	1.26	2	7
Porcupine	3.47	2.10	1	7
Porpoise #	2.94	1.65	2	7
Poultry	3.05	2.06	1	7
Prairie dog	3.94	1.85	1	7
Puffin	2.80	2.13	1	7
Puma	4.34	1.78	1	7
Rabbit	5.62	1.33	3	7
Racoon	3.40	2.04	1	7
Ram #	4.37	1.71	1	7
Rat	5.29	1.42	2	7
Raven	2.57	1.89	1	7
Reindeer	5.77	1.11	4	7
Rhesus monkey #	4.05	2.01	1	7
Rhinoceros	6.17	0.89	4	7
Roach	1.75	1.80	1	7
Robin	2.83	1.85	1	7
Rodent	3.08	2.21	1	7
Roe dear #	5.25	1.33	2	7
Rook	2.34	1.80	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	max
Rooster	2.87	2.09	1	7
Salamander	1.88	1.83	1	7
Salmon	2.42	1.90	1	7
Sardine	1.73	1.77	1	7
Sea lion	3.22	1.98	1	7
Seagull	2.45	2.06	1	7
Seahorse	1.75	1.97	1	7
Seal	3.32	2.02	1	7
Shark #	2.48	2.20	1	7
Sheep	5.10	1.42	1	7
Short tailed tit	1.87	1.89	1	6
Shrew	2.70	2.22	1	7
Shrimp	1.52	1.43	1	7
Siamese cat ~	4.24	1.78	1	7
Siberian tiger	4.85	1.54	1	7
Skate	1.73	1.54	1	7
Skunk	2.85	2.22	1	7
Skylark #	2.27	1.98	1	7
Sloth	2.51	2.39	1	7
Slug	1.36	1.61	1	7
Snail	1.40	1.84	1	7
Snake #	2.24	2.25	1	7
Sole	1.90	1.54	1	7
Sow #	4.30	1.83	1	7
Sparrow	2.48	2.03	1	7
Spider	1.55	1.98	1	7
Springbok #	3.46	2.13	1	7
Squid	1.86	1.86	1	7
Squirrel	4.09	1.79	1	7
Stag	5.55	1.37	2	7
Star fish	1.75	1.87	1	7
Starling	2.50	1.92	1	7
Stick insect	1.26	1.88	1	7
Stickleback	1.37	1.72	1	7
Stoat #	3.68	1.97	1	7
Sturgeon	2.01	1.75	1	7
Swan	3.29	1.87	1	7
Swift #	2.48	1.95	1	7
Sword fish	1.83	1.88	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Tapir #	2.49	2.45	1	7
Tarantula #	1.42	2.31	1	7
Tench	1.62	1.62	1	7
Thrush	2.71	1.93	1	7
Tiger	6.57	0.60	5	7
Toad	2.32	2.04	1	7
Tortoise	2.89	2.07	1	7
Trout	2.16	2.02	1	7
Tuna	2.16	2.02	1	7
Turkey	3.31	1.86	1	7
Turtle	3.25	1.83	1	7
Tyrannosaurus	2.60	2.48	1	7
Vole	3.53	2.04	1	7
Vulture	2.54	1.95	1	7
Wallaby #	4.95	1.46	3	7
Walrus	3.52	2.12	1	7
Warthog	3.67	2.00	1	7
Wasp	1.48	2.06	1	7
Water buffalo #	5.68	1.25	3	7
Water rat	3.07	2.09	1	7
Weasel	3.09	2.34	1	7
Whale	3.85	1.91	1	7
White tiger	5.45	1.38	3	7
Wild boar	4.19	1.86	1	7
Wild cat	4.57	1.76	2	7
Wild dog ~	3.89	1.92	1	7
Wildebeest #	4.89	1.67	2	7
Wolf	5.42	1.35	2	7
Wombat	2.51	2.39	1	7
Woodcock	2.17	1.84	1	7
Woodlouse	1.21	1.75	1	7
Woodpecker	2.83	1.93	1	7
Worm	1.45	1.87	1	7
Wren	2.84	1.97	1	7
Yak #	3.01	2.47	1	7
Yellow tit	2.08	1.51	1	6
Yellowhammer #	2.55	1.60	1	7
Yorkshire terrier	5.74	1.11	3	7
Zebra	6.07	0.99	4	7

Table C.6b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to the 61-70 age –category.

Fruit	**Mean**	**SD**	**Range**	
			min	**max**
Acorn	5.74	2.11	3	10
Almond	8.06	3.48	3	19
Apple	3.32	1.22	1	8
Apricot	5.53	3.08	1	14
Aubergine #	9.47	4.27	4	19
Avocado #	9.43	3.84	5	18
Banana	3.58	1.32	1	8
Berries	4.54	1.83	1	10
Bilberry #	5.55	2.46	2	12
Blackberry	5.55	2.46	2	12
Blackcurrant	4.88	2.90	2	15
Blueberry	6.34	3.48	3	17
Bramble #	7.14	4.99	3	18
Butternut squash	10.78	4.53	4	19
Cantaloupe melon	10.78	4.53	4	19
Cherry	5.07	1.63	2	8
Chestnut #	6.91	2.28	4	11
Citron	6.12	3.10	3	17
Clementine ~	6.12	3.10	3	17
Coconut	6.32	2.26	3	12
Cox apple # *	6.13	4.33	3	17
Crab apple	7.87	4.91	4	18
Cranberry	7.67	3.16	3	16
Cucumber #	4.68	2.23	1	10
Currant #	5.14	3.10	1	16
Damson #	9.14	4.38	4	19
Date	7.88	2.65	5	14
Dewberry #	9.31	3.99	4	19
Elderberry #	9.31	3.99	4	19
Fig #	7.37	3.44	3	16
Gala apple *	6.58	4.41	3	19
Galia melon # ~	10.59	4.14	4	19
Gooseberry #	7.86	3.62	5	19
Granny smith	5.96	3.84	3	15
Grapefruit	6.58	2.96	3	15

Fruit	Mean	SD	Range min	Range max
Grape	6.26	1.01	3	7
Green melon	3.03	2.23	1	7
Guava	2.17	2.66	1	7
Haw	1.41	1.53	1	6
Hazelnuts	1.61	2.18	1	7
Honeydew melon # ~	4.85	1.69	2	7
Horse chestnut	1.37	1.75	1	7
Jackfruit	1.53	2.90	1	7
Jaffa #	5.71	1.37	3	7
Kiwi	3.71	2.03	1	7
kumquat	1.96	2.47	1	7
Lemon	3.53	2.14	1	7
Lime	4.04	1.94	1	7
Loganberry #	3.42	2.05	1	7
Lychee # ~	2.89	2.40	1	7
Mandarin # ~	5.71	1.24	4	7
Mango	4.00	1.86	1	7
Melon	4.44	1.75	1	7
Nashi	1.14	2.27	1	7
Nectarine	5.69	1.28	4	7
Nuts # ~	1.64	2.33	1	7
Olive #	2.99	2.29	1	7
Orange	6.72	0.71	4	7
Papaya	2.70	2.38	1	7
Passion fruit	3.13	2.15	1	7
Paw paw #	2.14	2.57	1	7
Peach ~	6.28	1.04	3	7
Pear	6.05	1.20	2	7
Peppers	2.24	2.40	1	7
Persimmons	1.55	2.38	1	7
Pineapple	4.64	1.70	1	7
Plantain #	2.04	2.35	1	7
Plum	4.76	1.67	1	7
Pomelo	1.52	2.48	1	7
Pomegranate ~	3.55	2.03	1	7
Prune #	3.91	1.92	1	7
Quince	2.19	2.45	1	7
Raisin #	3.34	2.15	1	7
Raspberry	4.44	1.84	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	Range max
Red currant #	3.31	2.21	1	7
Redberry	2.17	2.34	1	7
Red grape	4.12	2.08	1	7
Rhubarb #	3.35	2.05	1	7
Rosehip # *	2.03	2.26	1	7
Satsuma ~	5.25	1.47	2	7
Sharon fruit ~	1.86	2.59	1	7
Sloe berry #	2.21	2.37	1	7
squash	2.07	2.37	1	7
Star fruit	2.20	2.39	1	7
Strawberry	6.33	1.04	3	7
Sultana	4.01	1.89	1	7
Tangerine	5.68	1.24	3	7
Tayberry	1.68	2.32	1	7
Tomato # *	2.76	2.41	1	7
Walnut #	1.61	2.18	1	7
Water melon	4.01	1.94	1	7
Whinberry	1.75	2.74	1	7
White currant #	1.94	2.67	1	7
White grape	4.04	2.10	1	7
Whortleberry	1.43	2.36	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Table C.7a. Mean, standard deviation (SD) and range (min-max) typicality values for animal words in individuals belonging to > 70 age –category.

Animal	Mean	SD	Range min	Range max
Aardvark	2.47	1.98	1	7
Adder	1.92	1.75	1	7
Albatross	1.94	1.87	1	6
Alligator	2.63	2.16	1	7
Alpaca #	2.56	2.15	1	7
Amoeba	1.17	0.97	1	4
Angel fish	1.58	1.69	1	7
Ant	1.83	2.54	1	7
Ant bear #	2.82	1.93	1	7
Anteater	2.41	2.46	1	7
Antelope #	3.77	2.04	1	7
Ape	3.84	2.20	1	7
Armadillo	2.10	2.31	1	7
Ass #	3.40	1.99	1	7
Baboon	3.28	2.31	1	7
Badger	4.13	1.88	2	7
Bald eagle	1.78	1.91	1	6
Bat	2.30	2.02	1	7
Bear	3.21	2.39	1	7
Beaver	2.90	2.24	1	7
Bee	2.06	2.40	1	7
Beetle	1.81	2.01	1	7
Bird #	2.18	2.46	1	7
Bison #	3.35	2.14	1	7
Blackbird	2.86	1.70	1	7
Blue bird	2.64	1.21	1	6
Blue tit	2.57	1.51	1	6
Boa constrictor	2.03	1.85	1	7
Boar #	3.44	2.11	1	7
Brontosaurus #	1.99	2.64	1	7
Brown bear	3.52	2.31	1	7
Buck #	3.14	2.27	1	7
Budgerigar	2.47	1.61	1	6
Buffalo	3.39	2.28	1	7
Bull	4.44	1.77	1	7

~ significant effect of age, # significant effect of gender, *significant effect of education

Animal	Mean	SD	Range min	max
Bullfinch # *	2.18	1.83	1	7
Bullock #	4.05	1.88	1	7
Butterfly	1.99	2.25	1	7
Buzzard	2.13	1.75	1	7
Calf ~	5.00	1.60	3	7
Camel #	3.93	2.07	1	7
Canary	2.82	1.51	1	6
Caribou #	3.15	2.35	1	7
Carp	2.20	1.30	1	6
Cat	5.95	1.29	3	7
Caterpillar	1.56	2.07	1	7
Cattle	5.23	1.59	2	7
Chaffinch	2.14	1.38	1	5
Chameleon	1.99	1.59	1	6
Cheetah	3.85	2.16	1	7
Chicken #	3.33	1.90	1	7
Chimpanzee	5.05	1.63	2	7
Chinchilla	2.64	2.30	1	7
Chipmunk	2.77	2.19	1	7
Clown fish	1.66	1.35	1	5
Cobra #	1.84	1.75	1	7
Cockatiel	1.96	1.56	1	7
Cocker spaniel ~	5.08	1.60	3	7
Cockerel	2.69	1.66	1	7
Cockroach	1.49	1.72	1	7
Cod	2.44	1.83	1	7
Condor #	2.22	1.89	1	7
Conger eel	1.92	1.20	1	5
Cougar #	3.02	2.35	1	7
Cow	5.87	1.33	3	7
Coyote	2.74	2.20	1	7
Cray fish	1.65	1.61	1	6
Crocodile	2.39	2.29	1	7
Crow	2.64	1.76	1	7
Cuckoo	2.22	1.50	1	6
Deer	4.28	1.76	1	7
Dingo #	2.68	2.30	1	7
Dog #	6.31	0.74	5	7
Dolphin	2.85	1.85	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Donkey	5.13	1.61	2	7
Dormouse	2.75	2.12	1	7
Dove	2.70	2.02	1	7
Dragon	1.79	2.17	1	7
Dragonfly	1.69	1.71	1	6
Dromedary #	2.81	2.31	1	7
Duck	3.41	1.83	1	7
Duckbill platypus	1.98	1.91	1	7
Eagle	2.57	2.01	1	7
Earthworm	1.56	2.26	1	7
Earwig	1.71	1.17	1	4
Echidna	1.46	1.54	1	5
Elephant	2.05	1.75	1	7
Eel	4.29	1.99	1	7
Elk #	3.28	2.08	1	7
Emu	1.93	1.54	1	6
Ewe #	4.26	1.71	1	7
Ferret	3.78	1.93	1	7
Field mouse	3.15	1.98	1	7
Finch	2.41	1.52	1	7
Fish #	1.94	2.43	1	7
Flamingo	2.76	1.56	1	7
Flea	1.43	1.92	1	7
Fly	1.75	2.27	1	7
Fowl	2.31	2.10	1	7
Fox	4.87	1.65	2	7
Frog #	2.55	1.53	2	7
Gazelle #	3.21	2.22	1	7
Gecko	1.97	0.89	1	4
Gerbil	2.59	2.46	1	7
Giant panda	3.48	2.22	1	7
Gibbon	2.60	2.31	1	7
Giraffe	4.34	2.13	2	7
Gnu #	2.05	1.96	1	7
Goldcrest	4.23	1.82	1	7
Goat	1.76	1.80	1	7
Goldfish	2.28	1.77	1	7
Gorilla	2.81	1.67	1	6
Greyhound	4.60	1.92	2	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Animal	Mean	SD	Range min	Range max
Grizzly bear	3.10	2.49	1	7
Groundhog	2.35	2.20	1	7
Guinea fowl	2.00	1.80	1	7
Guinea pig	3.06	2.13	1	7
Gull	2.52	1.32	1	5
Haddock	2.03	2.31	1	7
Hamster	4.12	1.91	2	7
Hare	3.50	2.13	1	7
Hart #	2.44	2.20	1	7
Hawk	2.10	1.73	1	7
Hedgehog	3.48	2.02	1	7
Heifer #	3.84	2.17	1	7
Hen	3.55	1.63	2	7
Heron	2.28	2.07	1	7
Herring	1.83	1.96	1	6
Hippopotamus	3.12	2.33	1	7
Hornet	1.71	1.44	1	6
Horse	6.55	0.74	5	7
Horsefly	1.27	1.12	1	5
Hyena	2.86	2.24	1	7
Ibex #	2.07	2.33	1	7
Iguana	2.01	1.82	1	7
Impala #	2.50	1.92	1	7
Insect	1.65	2.41	1	7
Invertebrate	1.28	1.53	1	5
Jack rabbit	2.47	2.22	1	7
Jackal #	2.74	2.27	1	7
Jackass #	2.86	2.18	1	7
Jackdaw #	2.14	1.53	1	6
Jaguar	3.94	2.13	1	7
Kangaroo	4.30	1.86	2	7
Kid #	3.19	2.02	1	7
Kitten	4.44	1.95	2	7
Kiwi	2.60	1.76	1	7
Koala	3.50	1.99	1	7
Koi carp	1.67	1.66	1	6
Komodo dragon	2.00	2.29	1	7
Lady bird	1.50	0.73	1	3
Lamb #	2.10	1.90	1	7

Animal	Mean	SD	Range min	Range max
Lemur	2.67	2.33	1	7
Leopard	3.93	2.16	1	7
Lion	5.47	1.52	3	7
Lizard	2.30	1.81	1	7
Llama #	2.60	2.58	1	7
Lobster	1.73	1.89	1	7
Long tailed tit	2.07	1.52	1	6
Lynx	3.31	1.96	1	7
Mackerel	2.09	1.44	1	5
Mammal	3.12	2.44	1	7
Manatee	1.66	1.75	1	6
Mandrill # *	2.45	2.24	1	7
Marmoset #	2.03	1.85	1	7
Marmot #	2.38	1.72	1	6
Marten #	2.50	2.02	1	7
Meerkat	2.46	2.18	1	7
Midge	1.51	1.84	1	6
Mink #	2.40	2.19	1	7
Manx	2.17	2.11	1	7
Mole	2.84	2.10	1	7
Mongoose #	2.85	1.99	1	7
Monkey #	3.14	2.37	1	7
Moose	3.10	2.25	1	7
Moth	1.70	1.60	1	7
Mouse ~	3.81	1.88	1	7
Mule	3.44	2.23	1	7
Musk ox #	2.46	2.61	1	7
Newt	1.83	1.18	1	5
Nightingale	1.93	1.32	1	4
Ocelot #	2.68	2.02	1	7
Octopus	2.05	1.91	1	7
Orang-utan	3.64	1.96	1	7
Oryx #	2.44	1.91	1	6
Ostrich	2.90	2.13	1	7
Otter #	3.43	2.13	1	7
Owl	2.70	1.45	1	6
Ox #	3.25	2.29	1	7
Panda	3.73	2.14	1	7
Panther	3.32	2.28	1	7

Animal	Mean	SD	Range min	Range max
Parakeet	1.87	1.60	1	6
Parrot	2.61	1.65	1	7
Partridge	2.42	1.70	1	7
Peacock	2.37	1.57	1	7
Peewit #	1.64	1.07	1	4
Pelican	2.46	1.71	1	7
Penguin ~	3.44	1.77	2	7
Perch	1.75	1.45	1	6
Pheasant	2.93	1.64	1	7
Pig	5.40	1.41	3	7
Pigeon	2.07	2.06	1	7
Piglet ~	3.94	1.95	1	7
Pike	1.84	1.75	1	7
Pine marten #	2.17	2.22	1	7
Piranha fish	1.50	1.79	1	7
Plaice	2.08	1.78	1	7
Platypus	2.04	2.47	1	7
Polar bear	3.30	2.34	1	7
Polar cat	2.41	2.52	1	7
Pony	5.49	1.39	3	7
Porcupine	2.65	2.16	1	7
Porpoise #	2.39	1.96	1	6
Poultry	2.56	2.31	1	7
Prairie dog	2.43	2.20	1	7
Puffin	2.55	1.04	1	5
Puma	3.23	2.12	1	7
Rabbit	5.08	1.63	3	7
Racoon	2.63	1.97	1	7
Ram #	4.13	1.83	1	7
Rat	3.48	2.08	1	7
Raven	1.86	1.70	1	7
Reindeer	4.27	1.80	1	7
Rhesus monkey #	3.15	2.25	1	7
Rhinoceros	3.11	2.34	1	7
Roach	1.97	1.74	1	7
Robin	3.01	1.98	1	7
Rodent	2.84	1.96	1	7
Roe dear #	3.40	2.15	1	7
Rook	2.21	1.73	1	7

Animal	Mean	SD	Range min	max
Rooster	2.68	1.91	1	7
Salamander	1.81	1.84	1	7
Salmon	2.38	2.11	1	7
Sardine	1.85	1.83	1	7
Sea lion	2.14	2.22	1	7
Seagull	2.44	1.94	1	7
Seahorse	1.34	1.89	1	7
Seal	2.57	2.01	1	7
Shark #	2.46	2.30	1	7
Sheep	5.75	1.33	4	7
Short tailed tit	1.68	0.95	1	4
Shrew	1.86	1.95	1	7
Shrimp	1.95	1.70	1	7
Siamese cat ~	3.45	2.14	2	7
Siberian tiger	2.65	2.58	1	7
Skate	1.85	1.33	1	5
Skunk	2.32	1.83	1	7
Skylark #	2.14	1.66	1	6
Sloth	2.31	2.33	1	7
Slug	1.55	2.11	1	7
Snail	1.45	2.27	1	7
Snake #	1.92	2.39	1	7
Sole	2.02	1.80	1	7
Sow #	4.73	1.70	2	7
Sparrow	2.50	2.14	1	7
Spider	2.18	2.26	1	7
Springbok #	3.48	1.95	2	7
Squid	1.49	1.63	1	6
Squirrel	4.62	1.72	2	7
Stag	3.83	2.07	1	7
Star fish	1.37	1.66	1	7
Starling	2.27	2.15	1	7
Stick insect	1.64	1.49	1	6
Stickleback	1.81	1.33	1	5
Stoat #	2.70	2.18	1	7
Sturgeon	2.36	1.38	1	5
Swan	3.19	1.61	1	7
Swift #	2.34	1.55	1	7
Sword fish	1.90	1.71	1	7

Animal	Mean	SD	Range min	Range max
Tapir #	1.78	1.99	1	7
Tarantula #	1.60	1.65	1	6
Tench	1.70	1.07	1	4
Thrush	2.54	1.64	1	7
Tiger	5.10	1.50	3	7
Toad	2.48	1.52	1	6
Tortoise	2.58	1.65	1	7
Trout	2.05	1.98	1	7
Tuna	2.72	1.56	1	6
Turkey	3.31	1.77	1	7
Turtle	2.58	1.42	1	6
Tyrannosaurus	2.04	2.91	1	7
Vole	2.32	1.89	1	7
Vulture	1.86	1.61	1	7
Wallaby #	3.10	2.02	1	7
Walrus	2.77	2.33	1	7
Warthog	2.45	2.37	1	7
Wasp	1.92	2.27	1	7
Water buffalo #	2.81	2.52	1	7
Water rat	2.72	2.26	1	7
Weasel	2.91	2.14	1	7
Whale	2.61	2.24	1	7
White tiger	2.83	2.43	1	7
Wild boar	2.82	2.29	1	7
Wild cat	2.49	2.53	1	7
Wild dog ~	2.75	2.38	1	7
Wildebeest #	3.44	2.08	1	7
Wolf	3.57	2.13	1	7
Wombat	1.84	2.16	1	7
Woodcock	1.89	1.73	1	7
Woodlouse	1.34	1.89	1	7
Woodpecker	2.13	1.68	1	7
Worm	1.89	2.08	1	7
Wren	2.41	1.69	1	7
Yak #	2.87	2.41	1	7
Yellow tit	1.70	1.45	1	6
Yellowhammer #	1.84	1.66	1	7
Yorkshire terrier	5.11	1.51	3	7
Zebra	3.95	2.07	1	7

Table C.7b. Mean, standard deviation (SD) and range (min-max) typicality values for fruit words in individuals belonging to > 70 age –category.

Fruit	Mean	SD	Range min	Range max
Acorn	1.47	1.80	1	6
Almond	3.34	1.64	1	7
Apple	6.71	0.60	5	7
Apricot	5.51	1.25	4	7
Aubergine #	3.68	1.92	1	7
Avocado #	3.55	2.07	1	7
Banana	6.55	0.74	5	7
Berries	4.82	1.66	2	7
Bilberry #	2.70	2.04	1	7
Blackberry	5.07	1.70	2	7
Blackcurrant	5.68	0.99	4	7
Blueberry	2.88	2.08	1	7
Bramble #	4.27	1.69	2	7
Butternut squash	2.91	2.24	1	7
Cantaloupe melon	3.29	2.15	1	7
Cherry	5.55	1.17	3	7
Chestnut #	3.23	1.49	1	6
Citron	2.87	2.13	1	7
Clementine ~	3.96	2.03	1	7
Coconut	3.30	2.21	1	7
Cox apple # *	5.83	1.31	3	7
Crab apple	2.76	2.14	1	7
Cranberry	3.84	1.86	2	7
Cucumber #	3.81	1.99	1	7
Currant #	4.62	1.46	2	7
Damson #	4.76	1.74	3	7
Date	3.97	1.77	2	7
Dewberry #	2.46	1.85	1	7
Elderberry #	3.48	1.74	1	7
Fig #	3.54	1.82	1	7
Gala apple *	3.81	2.05	1	7
Galia melon # ~	3.93	1.89	1	7
Gooseberry #	5.71	1.14	4	7
Granny smith	5.95	1.19	4	7
Grapefruit	5.10	1.66	2	7

Fruit	Mean	SD	Range min	Range max
Grape	6.36	0.65	5	7
Green melon	4.46	1.83	2	7
Guava	2.43	2.14	1	7
Haw	1.54	1.34	1	5
Hazelnuts	3.32	1.73	1	7
Honeydew melon # ~	4.29	1.78	1	7
Horse chestnut	1.91	2.12	1	7
Jackfruit	2.06	1.85	1	7
Jaffa #	5.34	1.38	3	7
Kiwi	4.62	1.50	3	7
kumquat	2.09	1.81	1	7
Lemon	4.30	1.74	1	7
Lime	3.65	1.94	1	7
Loganberry #	3.47	2.15	1	7
Lychee # ~	2.53	1.89	1	7
Mandarin # ~	5.22	1.54	3	7
Mango	4.21	2.05	2	7
Melon	5.55	1.32	3	7
Nashi	1.30	1.20	1	4
Nectarine	4.79	1.70	2	7
Nuts # ~	3.13	2.22	1	7
Olive #	4.28	1.64	2	7
Orange	6.65	0.61	5	7
Papaya	1.99	2.57	1	7
Passion fruit	2.79	2.13	1	7
Paw paw #	1.92	2.39	1	7
Peach ~	5.07	1.70	2	7
Pear	6.34	0.76	5	7
Peppers	4.78	1.35	3	7
Persimmons	1.70	1.23	1	5
Pineapple	5.53	1.38	3	7
Plantain #	1.73	2.43	1	7
Plum	6.11	0.99	4	7
Pomelo	1.74	1.63	1	6
Pomegranate ~	2.62	2.22	1	7
Prune #	3.59	2.07	2	7
Quince	2.97	1.75	1	7
Raisin #	4.95	1.40	2	7
Raspberry	4.40	1.72	1	7

~ significant effect of age, **#** significant effect of gender, *****significant effect of education

Fruit	Mean	SD	Range min	Range max
Red currant #	4.02	1.69	2	7
Redberry	2.24	2.34	1	7
Red grape	3.08	2.35	1	7
Rhubarb #	4.90	1.51	2	7
Rosehip # *	1.94	1.70	1	6
Satsuma ~	5.38	1.42	3	7
Sharon fruit ~	2.11	2.12	1	7
Sloe berry #	2.76	1.91	1	7
squash	2.89	2.18	1	7
Star fruit	1.96	2.07	1	7
Strawberry	5.92	0.92	4	7
Sultana	5.42	1.27	4	7
Tangerine	4.55	1.56	2	7
Tayberry	1.94	1.93	1	6
Tomato # *	5.74	1.21	3	7
Walnut #	2.98	1.96	1	7
Water melon	3.55	2.16	1	7
Whinberry	1.65	1.80	1	5
White currant #	2.47	2.16	1	7
White grape	5.55	1.12	4	7
Whortleberry	1.51	1.40	1	5

~ significant effect of age, **#** significant effect of gender, *****significant effect of education