KIMMO HATANPÄÄ

# Neuronal Dysfunction in Aging and Alzheimer's Disease

*University of Tampere*
*Tampere 1999*

# Neuronal Dysfunction in Aging and Alzheimer's Disease

*Acta Electronica Universitatis Tamperensis 2*

**ACADEMIC DISSERTATION**
University of Tampere,
Gerontology, School of Public Health
Finland

ISBN 951-44-4693-3
ISSN 1456-954X

# CONTENTS

## LIST OF ORIGINAL COMMUNICATIONS

This dissertation is based on the following original articles, which are referred to in the text by their Roman numerals. The articles are reproduced with the kind permission of the publishers. The impact factors of the journals are shown in parentheses as per Science Citation Index Journal Citation Report 1997.

I Hatanpää K, Brady DR, Stoll J, Rapoport SI, Chandrasekaran K. Neuronal activity and early neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1996; 40: 411-20. (9.5)

II Hatanpää K, Chandrasekaran K, Brady DR, Rapoport SI. No association between Alzheimer plaques and decreased levels of cytochrome oxidase subunit mRNA, a marker of neuronal energy metabolism. Brain Res Mol Brain Res 1998; 59: 13-21. (2.7)

III Hatanpää K, Isaacs KR, Shirao T, Brady DR, Rapoport SI. Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. J Neuropathol Exp Neurol 1999; 58: 637-43. (4.2)

IV Hatanpää K, Galdzicki Z, Chibber BA, Brady DR, Rapoport SI. Detergent-soluble amyloid β-peptide and neuronal function in Alzheimer disease and aging. Submitted, 1999.

## ABBREVIATIONS

| | |
|---|---|
| 12S rRNA | 12S ribosomal RNA |
| Aβ | Amyloid β-peptide |
| AD | Alzheimer's disease |
| DS | Down's syndrome |
| FAD | familial Alzheimer's disease |
| E-TB | early tangle-bearing |
| COX or CO | cytochrome oxidase |
| COX I-III | cytochrome oxidase subunits I-III |
| ISH | *in situ* hybridization |
| L-TB | late tangle-bearing |
| mtDNA | mitochondrial DNA |
| NFT | neurofibrillary tangle |
| NP | neuritic plaque |
| PD | Pick's disease |
| PET | positron emission tomography |
| $Poly(A)^+$ | polyadenylated mRNA |
| SP | senile plaque |
| TB | tangle-bearing |
| TF | tangle-free |

## ABSTRACT

Alzheimer's disease is the most common cause of dementia in the elderly. Clinically, neuronal dysfunction in Alzheimer's disease is evident on positron emission tomography scans, which demonstrate decreased energy metabolism in brains of patients with Alzheimer's disease. This decrease in brain energy metabolism is proportionally more severe and starts earlier than cerebral atrophy, suggesting that neuronal dysfunction cannot be completely explained by neuron loss.

Although remarkable progress has been made over the last few years in understanding the pathologic basis of Alzheimer's disease, the critical events leading to neuronal dysfunction, and eventually neuron death, are still not clear. Microscopic examination of Alzheimer brains reveals frequent neurofibrillary tangles, which are intraneuronal accumulations of abnormal cytoskeletal elements, as well as extracellular deposits called senile plaques, which consist of fibrillar amyloid β-peptide (Aβ). Although the presence of these two features is essential in confirming the diagnosis of Alzheimer's disease, their significance as factors contributing to neurodegeneration is controversial.

In the present study, sensitive methods were introduced for the detection and characterization of neuronal dysfunction in autopsy samples from Alzheimer brains. Gene expression of cytochrome oxidase, an enzyme of mitochondrial oxidative phosphorylation, was used as a marker of energy metabolism in individual neurons. It was found that formation of neurofibrillary tangles is associated with progressive loss of cytochrome oxidase mRNA. Interestingly, compared with control tangle-free neurons, Alzheimer's disease tangle-free neurons also showed a decrease in cytochrome oxidase mRNA. In contrast, senile plaque density, vicinity of neurons to senile plaques, and levels of nonfibrillar Aβ did not correlate with cytochrome oxidase mRNA or cytochrome oxidase enzyme activity. In addition to cytochrome oxidase, other markers of neuronal function were quantified. Levels of drebrin, a marker of synaptic plasticity, were found to decrease markedly during normal aging. An additional 81 % decrease was demonstrated in Alzheimer brains. This is in contrast to

cytochrome oxidase, which decreases in Alzheimer's disease but not in normal aging. These results show that neuronal dysfunction associated with normal aging can be demonstrated using biochemical and molecular methods. The impairments that occur during normal aging are much less severe than in Alzheimer's disease. In addition, the results indicate that whereas impairments in neuronal plasticity may occur in both aging and Alzheimer's disease, only Alzheimer's disease is characterized by a marked decrease in neuronal energy metabolism.

# 1. INTRODUCTION

In 1903, Bielschowsky improved the silver stain originally discovered by Golgi in 1873, making it possible for the first time to clearly visualize cytoskeletal components of neurons [Berchtold and Cotman, 1998]. A "hot new method" at the time, it was quickly adopted by Alois Alzheimer, who used it to detect a startling new type of pathology in the brain of a woman who died a few years after developing dementia at age 51. In his case report that he presented at a meeting in 1906 and published in 1907, Alzheimer described numerous intraneuronal neurofibrillary tangles as well as the widespread presence of extracellular deposits of a peculiar substance [Alzheimer, 1987], now known to consist primarily of amyloid β-peptide (Aβ). The clinical presentation and neuropathological features of this case stood out from the cases he had examined previously, most of which were caused by neurosyphilis or vascular dementia, leading Alzheimer to suggest that the case might represent a previously undescribed disease.

In 1911, Alzheimer published a more comprehensive paper, presenting an additional case and discussing case reports that had been published by others after his initial paper [Alzheimer, 1991; Moller and Graeber, 1998]. Many of those case reports were already referring to the condition as "Alzheimer's disease". The most important endorsement, however, had come in 1910, when "Alzheimer's disease" was mentioned in the new edition of the *Textbook of Psychiatry* by Emil Kraepelin, the chairman of Alzheimer's department and the foremost psychiatrist in the world at the time [Berchtold and Cotman, 1998]. According to Kraepelin's definition, Alzheimer's disease referred to presenile dementia, dementia occurring at an unusually early age, whereas dementia of old age was called senile dementia. By that time, however, it had become clear to Alzheimer that the clinical and neuropathological features of presenile and senile dementia were very similar, if not identical, except for the age of presentation. In his 1911 paper, Alzheimer argued that presenile and senile dementia should be considered two forms of the same disease [Alzheimer, 1991]. Today, most investigators agree with Alzheimer's position in this issue, although Kraepelin's view

still has supporters as well, particularly in Europe [Blass, 1996; Svennerholm and Gottfries, 1994]. During the last decade, the discovery of specific genes associated with Alzheimer's disease has provided scientific basis for the new dichotomy of familial versus sporadic Alzheimer's disease.

By careful examination of his silver stained tissue sections, Alzheimer was able to make a number of suggestions regarding the pathogenesis of the disease. Remarkably, many of his suggestions have been "rediscovered" and confirmed to be correct by modern methods during the last three decades. These include Alzheimer's suggestion that neurofibrillary tangles originate from modified cytoskeletal elements [Alzheimer, 1991], a view which was opposed by Bielschowsky, among others [Berchtold and Cotman, 1998]. Alzheimer also pointed out the dissimilarity of the anatomical distributions of neurofibrillary tangles and senile plaques [Alzheimer, 1991]. He correctly inferred that plaques result from active deposition rather than from disintegration of cells [Alzheimer, 1991]. He noted that not all brain regions are equally affected by the disease process, and observed the relative absence of atrophy in the primary motor and the primary sensory cortices [Alzheimer, 1991; Moller and Graeber, 1998]. Moreover, he correctly described the various stages neurofibrillary degeneration, which ultimately lead to neuronal death and disappearance except for the persistent "ghost tangles" [Alzheimer, 1991; Berchtold and Cotman, 1998].

One suggestion by Alzheimer that is still controversial is his opinion that senile plaques are not the cause of the disease but only an accompanying feature of the degenerative process. He based his conclusion on the observations that plaques seemed to dislocate rather than destroy brain tissue, degenerative changes were present in brain regions where plaques were not found, and that there were cases of Alzheimer's disease with very few plaques [Alzheimer, 1991; Davis and Chisholm, 1999].

Even today, there is still no agreement as to the neuropathologic substrate of dementia in Alzheimer's disease. Popular theories include those focusing on senile plaques, neurofibrillary tangles, synapse loss, neuron death, and degeneration of specific neurotransmitter systems such as the cholinergic system. However, identification of

specific mutations associated with familial Alzheimer's disease and recent progress in characterizing the immediate cellular effects of these mutations has generated optimism and expectations that it may be possible to unravel the pathologic cascade starting from its initial steps [Price *et al*., 1998b].

Alzheimer did not directly address normal cerebral aging in his two papers. Even in the modern scientific literature, normal cerebral aging has received relatively little attention. Although it is generally accepted that normal aging is associated with some cognitive impairment, this is often disregarded as insignificantly mild and labeled as "benign" [Flicker *et al*., 1991]. Therefore, recent neurological and neuropathological research has largely focused on the distinction between normal cerebral aging and dementia, with the object of identifying persons with dementia as early and reliably as possible [Blass, 1996]. However, it has also been argued that age-associated cognitive impairment may not be as insignificant as previously thought and that the use of clinical tests designed for dementia screening may overlook the cognitive impairment associated with normal aging [Crook and Ferris, 1992]. In any case, given that aging is the most important risk factor of Alzheimer's disease, better understanding of the degenerative changes that occur in aging would likely contribute to the development of therapeutic approaches for the prevention of Alzheimer's disease and related dementias.

In the present study, neuronal dysfunction in aging and Alzheimer's disease was studied using histochemical, biochemical, and molecular biological methods. The results suggest that although normal aging is clearly associated with progressive neuronal dysfunction, this dysfunction is much less severe than in Alzheimer's disease. In addition, it was found that whereas impairments in neuronal plasticity may occur in both aging and Alzheimer's disease, only Alzheimer's disease is characterized by a marked decrease in neuronal energy metabolism. The extent of the decrease in energy metabolism appears not to be directly related to Aβ burden in Alzheimer's disease. In contrast, it was found that neurodegeneration associated with formation of neurofibrillary tangles accounts for some, although probably not for all, of the decrease in neuronal energy metabolism.

# 2. REVIEW OF THE LITERATURE

## 2.1. Normal aging of the brain

It is unfortunately true that at least to some extent cognitive functions do decline with normal aging [Blass, 1996; Unger *et al*., 1999]. A carefully conducted longitudinal study demonstrated deterioration in one or more cognitive abilities in 100 % of subjects by age 70 years (Fig. 1) [Schaie, 1989]. This analysis did not include memory among the cognitive abilities that were examined, although memory may be the cognitive ability that is most consistently impaired with aging. The types of memory requiring neocortical association areas, such as memory for temporal order (recency memory), are more vulnerable to age-associated impairment than others, such as recognition memory [Fabiani and Friedman, 1997]. In general, components of the crystalline intelligence remain preserved or even increase until late age, while fluid intelligence decreases [Schaie, 1994]. This means that elderly people act successfully in routine situations, but impairments become evident in flexible adaptation to new situations and problem solving [Mielke *et al*., 1998].

Due to differences in the level of cognitive ability in young adulthood and in the rate of subsequent decline, old persons display a wide spectrum of cognitive abilities, ranging from normal to mild cognitive impairment, and to dementia. A number of terms have been used to describe the middle group, including benign senescent forgetfulness, age-associated memory impairment, age-consistent memory impairment or late-life forgetfulness, cognitively impaired not demented, and mild cognitive impairment (for references, see [Mufson *et al*., 1999]). It is beyond the scope of this thesis to discuss the definitions of these terms in detail, but in general, they all refer to individuals whose cognitive function is below the level considered normal, but who do not meet the clinical criteria for dementia. Difference of opinion exists on whether normal levels of cognitive performance for old persons should be defined using age standardized normal values or those of young adults [Crook and Ferris, 1992].

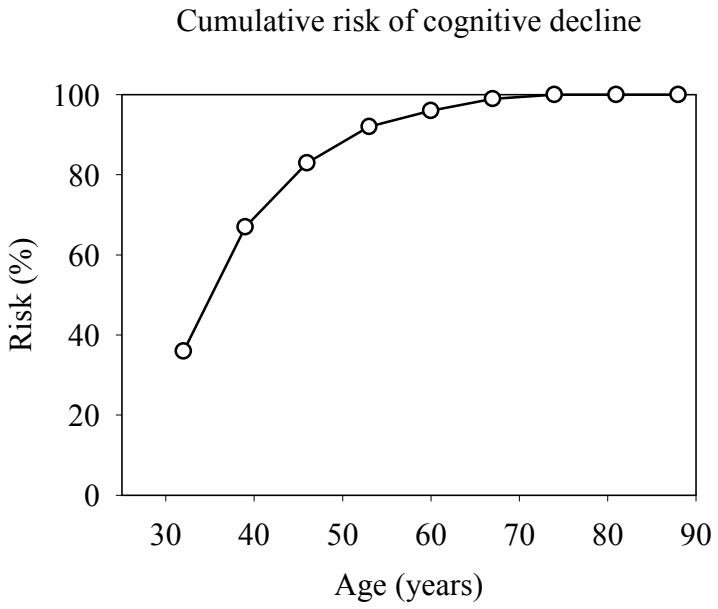



**Fig. 1.** Cumulative risk of cognitive decline in one or more cognitive abilities, as estimated on the basis of a longitudinal study. The cognitive abilities that were examined included verbal meaning, spatial orientation, inductive reasoning, number addition, and word fluency. Memory, which is the most sensitive cognitive ability for aging effects, was not included. Redrawn from [Schaie, 1989].

Since Alzheimer described the disease that bears his name, distinguishing it from normal aging, the trend in scientific literature, as well as in the popular press, has been the widening of the concept of abnormal cerebral aging. What was discounted as "benign senescent forgetfulness" in the 1960's [Crook and Ferris, 1992] has become an object of intensifying scientific study. This trend will undoubtedly continue, because the proportion of the elderly in society is increasing, as is the political weight of their interests. Other factors supporting this trend include advances in medicine allowing the elderly to stay physically active longer, economic growth providing opportunities to stay in the workforce past the usual age of retirement, advances in automation of physical labor, and advances in information technology. All these factors have created a demand for the elderly to maintain their mental acuity at a much higher level than what was previously thought to be necessary. In the future, any cognitive decline relative to the individual's previous performance will likely be considered pathological and sufficient indication for pharmacological intervention. As pointed out by Crook

and Larrabee [Crook and Larrabee, 1988], patients tend to complain that their memory abilities have declined from what they once were; they rarely complain that their abilities are worse than those of the average person 60, 70, or 80 years of age.

The pathologic basis of age-associated cognitive impairment is not clear. For decades, widespread neuronal death and synapse loss were considered to be inevitable concomitants of normal aging of the human brain. However, recent post-mortem studies using modern stereology suggest that the extent of neuronal death, at least in the neocortical and hippocampal regions studied so far, is restricted in normal aging and unlikely to account for age-related impairment of neocortical and hippocampal functions [Morrison and Hof, 1997]. Even the widely held notion of synapse loss during normal aging has been challenged [Scheff *et al*., 1997]. Nevertheless, in vivo mapping of cholinergic synapses using positron emission tomography has demonstrated that the number of cholinergic synapses, which represent 6-7 % of all synapses, declines 3.7 % per decade [Kuhl *et al*., 1999]. In addition, aging is associated with structural changes in the dendritic arbor, as is evident in increased average length of terminal dendrites [Arendt *et al*., 1995] and in marked loss of dendritic spines [Catala *et al*., 1988; Jacobs *et al*., 1997]. Positron emission tomography studies have demonstrated a small but significant decline in the overall cerebral glucose uptake with age, with association neocortical areas showing the most prominent reductions [Grady *et al*., 1998; Petit-Taboue *et al*., 1998]

Mild Alzheimer pathology (senile plaques and neurofibrillary tangles) is a common finding in brains of nondemented elderly individuals [Braak and Braak, 1997; Dickson *et al*., 1992]. Some studies have suggested that age-associated cognitive impairment could result from mild Alzheimer pathology in the form of neocortical plaques [Morris *et al*., 1991; Morris *et al*., 1996]. However, other studies have demonstrated that some neuropsychologically normal people, including people who have been prospectively studied for more than a decade and to within a few months before death, have numerous senile plaques in their neocortex [Delaere *et al*., 1990; Dickson *et al*., 1992]. One study reported a strong correlation between entorhinal cortex plaque load and cognitive status (MMSE score) [Cummings *et al*., 1996], whereas no correlation at all

was found between the two in a similar, more recent study [Mufson *et al*., 1999]. Furthermore, Alzheimer pathology, except for occasional neurofibrillary tangles in the entorhinal cortex [Braak and Braak, 1997], is extremely rare in persons younger than 50 years. In contrast, age-associated cognitive decline relative to the individual's past performance, even excluding memory impairment, can be demonstrated in more than 80 % of individuals by age 50 (Fig. 1). In conclusion, there is conflicting evidence regarding the role of Alzheimer pathology in age-associated cognitive impairment, but it is probably safe to conclude that Alzheimer pathology is not the sole pathogenic factor underlying age-associated cognitive impairment.

### 2.2. Alzheimer's disease

Alzheimer's disease is the most common cause of dementia and affects 15 % of people over age 65. Clinically, Alzheimer's disease is characterized by progressive dementia. Positron emission tomography studies have demonstrated decreases in regional cerebral metabolic rate, which correlate significantly with dementia severity [Berent *et al*., 1999; Pietrini *et al*., 1999; Rapoport, 1991; Rapoport *et al*., 1996]. These decreases in energy metabolism remain significant even after correction for brain atrophy, indicating that the decreases reflect neuronal dysfunction rather than simply neuron loss [Ibanez *et al*., 1998]. Neuropathologically, Alzheimer's disease is characterized by selective damage to brain regions and neural circuits critical for cognition and memory, including neocortical association areas, the hippocampus, amygdala, and basal forebrain cholinergic system [Jellinger and Bancher, 1998]. Affected neurons accumulate neurofibrillary tangles, which principally consist of abnormally phosphorylated tau, a microtubule-binding protein. In addition to neurofibrillary tangles, which are intracellular, there are frequent extracellular deposits called senile plaques. These are primarily composed of amyloid fibrils, which are highly insoluble aggregations of Aβ, a 4 kD peptide derived from β-amyloid precursor protein [Selkoe *et al*., 1986; Wisniewski *et al*., 1997].

Synapse loss is a prominent and early feature in Alzheimer's disease [Heinonen *et al*., 1995; Scheff *et al*., 1990; Scheff and Price, 1993]. Significant inverse correlations have been reported between synapse counts and dementia severity [DeKosky and Scheff, 1990; DeKosky *et al*., 1996]. There is no spatial relationship between the distribution of synapse loss and senile plaques [Heinonen *et al*., 1995], suggesting that synapse loss is not caused by senile plaques. It has been argued that degeneration and loss of synapses constitute the pathologic substrate of cognitive impairment [DeKosky *et al*., 1996] and decreased cerebral energy metabolism [Chandrasekaran *et al*., 1996; Rapoport *et al*., 1996] in Alzheimer's disease.

Remarkable progress has been made over the last few years in the molecular genetics of Alzheimer's disease, leading to identification of three genes whose mutations cause familial, early-onset, autosomal dominant Alzheimer's disease. These genes are the genes encoding for amyloid precursor protein (located in chromosome 21), presenilin-1 (chromosome 14) [Crook *et al*., 1997; Crook *et al*., 1998; Haltia *et al*., 1994; Perez-Tur *et al*., 1996], and presenilin-2 (chromosome 1) [Price *et al*., 1998b]. Interestingly, all familial Alzheimer's disease-associated mutations identified so far affect the processing of amyloid precursor protein in such a way that production of the long, highly amyloidogenic form of Aβ peptide (Aβ42/43) is increased [Mann *et al*., 1996; Price *et al*., 1998b]. These findings have provided support for the so-called amyloid cascade hypothesis, which states that aggregation of Aβ into fibrils and their deposition in senile plaques are critical in the pathogenesis of Alzheimer's disease [Hardy, 1997; Selkoe, 1994; Wisniewski *et al*., 1997].

So far, no specific gene mutations causing late-onset Alzheimer's disease, which is much more common than familial Alzheimer's disease, have been identified. However, certain alleles of apolipoprotein E (APOE) and a2 macroglobulin (A2M) are associated with an increased risk for Alzheimer's disease. Interestingly, these two genes appear to confer risk for Alzheimer's disease in different ways: APOE-ε4 allele lowers the age of onset in people who are vulnerable to Alzheimer's disease, whereas A2M-2 allele confers vulnerability to Alzheimer's disease [Price *et al*., 1998a]. The mechanisms by which these two genes interact with the pathogenesis of Alzheimer's disease is yet to

be discovered; however, as both APOE and A2M bind Aβ, these proteins may be involved in clearance of Aβ from the brain [Blacker *et al*., 1998; Wisniewski *et al*., 1997].

Several transgenic mouse models of Alzheimer's disease have been generated. These mice express human amyloid precursor protein and presenilin genes with familial Alzheimer's disease-associated mutations. They show senile plaque-like extracellular deposits of Aβ. However, so far, the full spectrum of neuropathologic features characteristic of human Alzheimer's disease has not been reproduced [Riekkinen *et al*., 1998]. For example, although phosphorylated tau is present in dystrophic neurites associated with the Aβ deposits, neurofibrillary tangles have not been described [Price *et al*., 1998b]. There is also no neuronal loss in these mice [Hyman *et al*., 1997; Irizarry *et al*., 1997a; Irizarry *et al*., 1997b]. Progressive learning and memory impairments have been described in a mouse overexpressing human amyloid precursor protein with a familial Alzheimer's disease-mutation and in a mouse expressing both mutated amyloid precursor protein and presenilin [Holcomb *et al*., 1998]. Interestingly, these impairments become evident before the appearance of senile plaques in the brains of these mice [Holcomb *et al*., 1998]. One possible explanation is that Aβ may initially accumulate in diffusible or membrane-associated forms, which cannot be detected histologically.

Therapeutic agents that affect the concentration, deposition, aggregation, degradation, clearance, or toxicity of Aβ are under development [Christie *et al*., 1999; Howlett *et al*., 1999; Vasan *et al*., 1996; Wood *et al*., 1996]. At present, we do not know which of these approaches will prove to be effective. It is also possible that other consequences of abnormal processing of amyloid precursor protein, rather than extracellular aggregation and deposition of fibrillar Aβ, are critical in the pathogenetic cascade [Neve, 1996]. Several mechanisms for the toxicity of nonfibrillar Aβ have been suggested. Low concentrations of monomeric Aβ induce leakage of choline, the precursor of acetylcholine, from cultured neuronal cells [Allen *et al*., 1997; Ehrenstein *et al*., 1997; Galdzicki *et al*., 1994]. Another possibility is that Aβ exerts its toxicity in

the cytoplasm of neurons. In cultured neurons, the site of Aβ production is intracellular, in the endoplastic reticulum [Cook *et al*., 1997], whereas in nonneuronal cells Aβ is produced at the cell surface [Hartmann *et al*., 1997]. Aβ has also been demonstrated to be present in the cytoplasm of neurons in Alzheimer's disease and its presence has been associated with neuronal damage [LaFerla *et al*., 1997; Rosenblum, 1999; Troncoso *et al*., 1996]. In addition, it has been shown that certain molecules found in the brain can chaperone the *in vitro* self-association of Aβ into diffusible, nonfibrillar oligomers, which are potent neurotoxins in cell culture [Lambert *et al*., 1998]. Their toxicity was specific to neurons (astrocytes were unaffected) and was seen at nanomolar concentrations. In contrast, fibrillar Aβ did not cause cell death even at micromolar concentrations [Lambert *et al*., 1998]. Recently, evidence has emerged showing that familial Alzheimer's disease-associated mutations cause intracellular accumulation of carboxyterminal fragments of amyloid precursor protein other than Aβ and that these fragments are neurotoxic [Islam and Levy, 1997; Kim *et al*., 1999a; McPhie *et al*., 1997; Suh, 1997]. Discoveries such as these may help to explain the weak or nonexistent correlations found in most studies between senile plaque density and dementia severity and between senile plaque density and neuron loss [Jellinger and Bancher, 1998; Swaab *et al*., 1998]. It is also interesting that both "plaque-predominant" (few or no neurofibrillary tangles) and "neurofibrillary tangle-predominant" (few or no senile plaques) forms of Alzheimer's disease have been described [Jellinger and Bancher, 1998; Swaab *et al*., 1998], indicating that neither senile plaques nor neurofibrillary tangles are essential features of Alzheimer's disease.

## 2.3. Molecular markers of neuronal function

### 2.3.1. Cytochrome oxidase

Cytochrome oxidase is one of the most complex proteins found in living organisms. In mammals, it consists of 13 subunits, the 3 largest of which are encoded by the mitochondrial DNA (Fig. 2), whereas the remaining subunits are encoded by the nuclear DNA. Cytochrome oxidase is part of the mitochondrial oxidative phosphorylation enzyme chain, which produces ATP. As the terminal enzyme of the chain, it receives electrons from cytochrome c and transfers them to oxygen and hydrogen ions, forming water. It uses the energy that is released to pump protons (hydrogen ions) across the inner mitochondrial membrane to generate a proton gradient. This gradient is then used by the ATP synthase to synthesize ATP (Fig. 3). [Gennis and Ferguson-Miller, 1995]

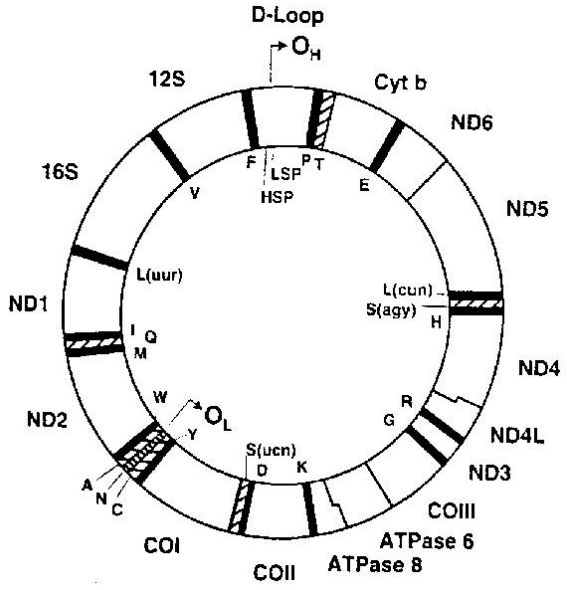



**Fig. 2.** Diagram of human mitochondrial DNA. Redrawn from [Sherratt *et al*., 1997].

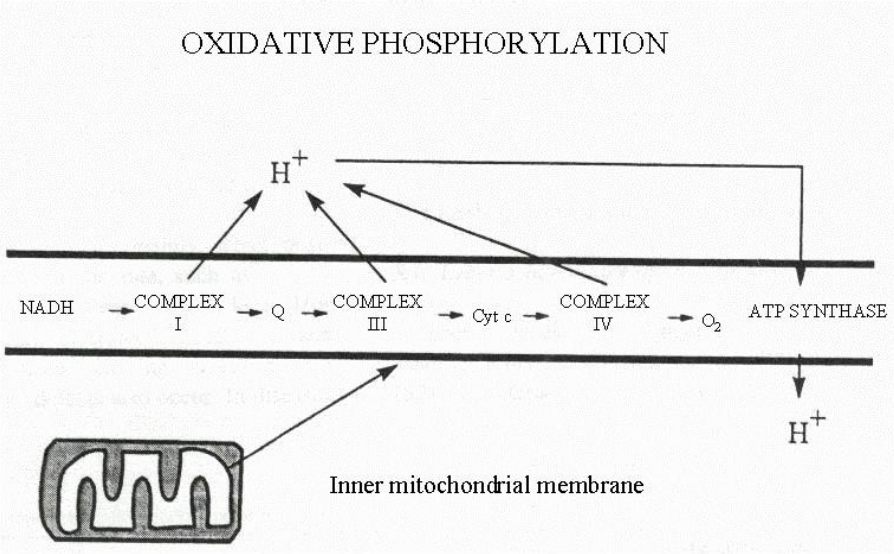



**Fig. 3.** Schematic presentation of mitochondrial oxidative phosphorylation. Cytochrome oxidase is complex IV of the oxidative phosphorylation chain (respiratory chain). It donates electrons to oxygen to form water. At the same time, it pumps protons across the mitochondrial inner membrane to create a proton gradient, which is used by the ATP synthase to synthesize ATP

Cytochrome oxidase accounts for more than 90 % of oxygen consumption by living organisms on earth. It is essential for organs like the kidney, heart, and brain, which depend on oxidative metabolism. Although the brain represents only 2 % of the total body weight of an adult human, it consumes more than 20 % of the total oxygen intake of the body. Neurons derive almost all of their energy from oxidative phosphorylation. They use ATP for ion pumping to maintain the resting membrane potential, for axoplasmic transport, and the synthesis of macromolecules and neurotransmitters. Of these, maintenance of ion balance constitutes the major energy consuming function of neurons. Increased synaptic firing promotes increased oxidative phosphorylation to generate ATP, which is used by the $Na^+/K^+$ ATPase to restore the resting membrane potential. [Chandrasekaran *et al*., 1996; Rapoport *et al*., 1996; Wong-Riley, 1989b]

The critical role of cytochrome oxidase in neuronal energy metabolism and the continuous dependence of neurons on oxidative phosphorylation to maintain synaptic firing led Margaret Wong-Riley to propose in the 1970s that the level of cytochrome oxidase enzyme activity should correlate with neuronal activity [Wong-Riley, 1989a].

Since then, her laboratory has played a central role in demonstrating a relationship between cytochrome oxidase enzyme activity and neuronal activity in various brain regions and neuronal groups under normal conditions, as well as in demonstrating adaptive adjustment of cytochrome oxidase activity in response to experimentally induced changes in neuronal activity.

Throughout the brain, gray matter manifests much higher cytochrome oxidase activity than white matter. In addition, the neuronal groups that exhibit high levels of spontaneous synaptic firing, such as the brain stem auditory relay nuclei, basal ganglia, thalamus, and CA3 of the hippocampus, are rich in cytochrome oxidase [Difiglia *et al*., 1987; Jones *et al*., 1986; Kageyama and Wong-Riley, 1982; Nomura and Mizuno, 1986; Wong-Riley, 1976; Wong-Riley *et al*., 1978]. Cytochrome oxidase enzyme histochemistry contributed to the discovery of metabolically active zones, known as "puffs" or "blobs", involved in color processing in the primate visual cortex [Carroll and Wong-Riley, 1984; Tootell *et al*., 1988].

At the cellular level, highest cytochrome oxidase enzyme activity is at sites of high energy demand, especially at postsynaptic terminals of excitatory synapses [Borowsky and Collins, 1989b]. Cytochrome oxidase enzyme activity is sensitive to neuronal metabolic demands. For example, chemical or surgical deafferentation decreases cytochrome oxidase activity [Borowsky and Collins, 1989a; Deyoe *et al*., 1995; Fujii *et al*., 1993; Hevner and Wong-Riley, 1993; Hevner and Wong-Riley, 1991; Wong-Riley, 1979; Wong-Riley, 1989a]. The reductions can be prevented by electrical stimulation of the postsynaptic neurons [Wong-Riley *et al*., 1981].

There are no previous studies on cytochrome oxidase enzyme activity in the human brain during aging. However, in rat cerebral cortex, cytochrome oxidase enzyme activity and mRNA have been reported to either remain unchanged or to increase slightly in aging [Gorini *et al*., 1989; Nicoletti *et al*., 1998; Villa and Gorini, 1991].

Cytochrome oxidase is decreased in Alzheimer's disease brain regions that typically demonstrate neuropathology, such as midtemporal association neocortex, but are not

changed in the relatively unaffected primary sensory and motor cortices [Chandrasekaran *et al*., 1994; Hatanpää *et al*., 1996; Mutisya *et al*., 1994; Simonian and Hyman, 1994a; Simonian and Hyman, 1994b]. The distribution of decreased cytochrome oxidase activity and mRNA in the postmortem Alzheimer's disease brain corresponds to decreased glucose metabolism [Rapoport, 1991] measured in life. Therefore, cytochrome oxidase appears to be useful as an indicator of impaired neuronal function in Alzheimer's disease.

### 2.3.2. Drebrin

Drebrin is an actin-binding protein which was first isolated from embryonic chicken brains by Tomoaki Shirao while he was a postdoctoral student in the laboratory of Professor Obata [Shirao and Obata, 1985; Shirao and Obata, 1986]. Professor Obata wanted to name the new protein 'embrin' because of its high expression in the embryonic brain. However, Dr. Shirao objected to his supervisor's decision. He believed the new protein might turn out to be important in the adult brain as well, so 'embrin' might become misleading. As a compromise, the protein was finally named 'drebrin', which was formed by combining the words 'dendrite', indicating the location of drebrin in the adult brain, and 'embryo' (T. Shirao, personal communication).

In the rat and human brains, two isoforms of drebrin, generated by alternate splicing of the mRNA, have been identified: drebrin E and drebrin A [Asada *et al*., 1994; Shirao *et al*., 1989; Toda *et al*., 1993]. Drebrin E is the predominant isoform during development, whereas drebrin A is the predominant isoform in the adult rat brain, where only trace amounts of drebrin E are present [Shirao, 1995]. In the human brain, the ratio of drebrin A to drebrin E rises from less than 1 to about 2 between the ages of 0 and 60 years, and decreases to about 1 between the ages of 60 and 95 years (Hatanpää, unpublished). Both drebrin isoforms show similar actin binding properties *in vitro*. The functional significance of the existence of these two isoforms is not known. In the adult brain, drebrin is mainly localized in dendritic spines, which are postsynaptic terminals of excitatory synapses. Drebrin modulates dendritic morphology

by regulating actin polymerization [Hayashi *et al*., 1996; Hayashi and Shirao, 1999; Shirao, 1995; Shirao and Obata, 1992; Toda *et al*., 1999]. In Alzheimer's disease cerebral cortex and the hippocampus, drebrin levels show marked reductions, which are proportionally more severe than the reductions in the levels of the presynaptic protein synaptophysin [Harigaya *et al*., 1996; Hatanpää *et al*., 1999], probably reflecting degenerative changes and functional impairments that occur in postsynaptic terminals.

Morphometric studies using the Golgi impregnation method have provided interesting insight into dendritic plasticity. In normal aging, the terminal segments of dendrites grow, increasing the size of the dendritic tree [Arendt *et al*., 1995]. This may be a compensatory response to age-associated impairments in synaptic signal transduction. In contrast, Alzheimer's disease is associated with an aberrant pattern of dendritic growth, where growth and branching of dendrites is largely limited to the proximity of the cell body. The result is an increased dendritic density within the dendritic tree without an increase in its size [Arendt *et al*., 1995]. It is tempting to speculate that disturbed mechanisms regulating dendritic plasticity may lead to Alzheimer's disease by misdirecting dendritic growth, thereby preventing sufficient compensation for the age-related impairments in synaptic signal transduction.

## 3. GENERAL QUESTION AND SPECIFIC AIMS

The general question addressed by this study is: What is the pathologic basis of neuronal dysfunction in aging and Alzheimer's disease?

The specific aims of this study are:

1) Quantify neuronal energy metabolism at the level of individual Alzheimer's disease neurons, using cytochrome oxidase mRNA as a marker, to examine possible association between decreased neuronal energy metabolism and the two major pathologic features of Alzheimer's disease: neurofibrillary tangles and senile plaques (I, II)
2) Compare cytochrome oxidase mRNA levels in Alzheimer's disease and control neurofibrillary tangle-free neurons to determine if apparently intact neurons in Alzheimer brains also show decreased cytochrome oxidase mRNA (I)
3) Determine changes in levels of energy metabolic markers, such as cytochrome oxidase, synaptic plasticity markers, such as drebrin, and structural proteins, such as β-tubulin, during normal aging to characterize age-associated neuronal dysfunction (III).
4) Compare levels of energy metabolic markers, synaptic plasticity markers, and levels of structural proteins in Alzheimer's disease and control brains to elucidate the mechanisms of neurodegeneration in Alzheimer's disease (III).
5) Quantify levels of nonfibrillar Aβ peptide in Alzheimer brains and correlate these values with levels of energy metabolic and synaptic markers to examine the possible role of nonfibrillar Aβ in neuronal dysfunction (IV)

# 4. MATERIALS AND METHODS

## 4.1. Subjects

Brain samples for this study (I-IV) were collected at autopsy and kept frozen at –80° C until needed. The subjects were chosen based on availability of frozen tissue from the brain regions of interest, which were the midtemporal association neocortex (I-IV) and the primary motor cortex (Fig. 4; I-II). A short postmortem interval was an additional criterion. When more tissue became available in the course of this study, it was possible to shorten the arbitrary limit on postmortem interval, which was originally set at 20 h (I), then shortened to 15 h (II), and finally to < 10 h (III-IV). No variable that was quantified showed a significant or near-significant ($p < 0.1$) correlation with postmortem interval in controls and Alzheimer's disease cases considered separately or combined, indicating that postmortem degradation did not confound this study (Fig. 5). The average postmortem interval was not statistically different between Alzheimer and control brains.

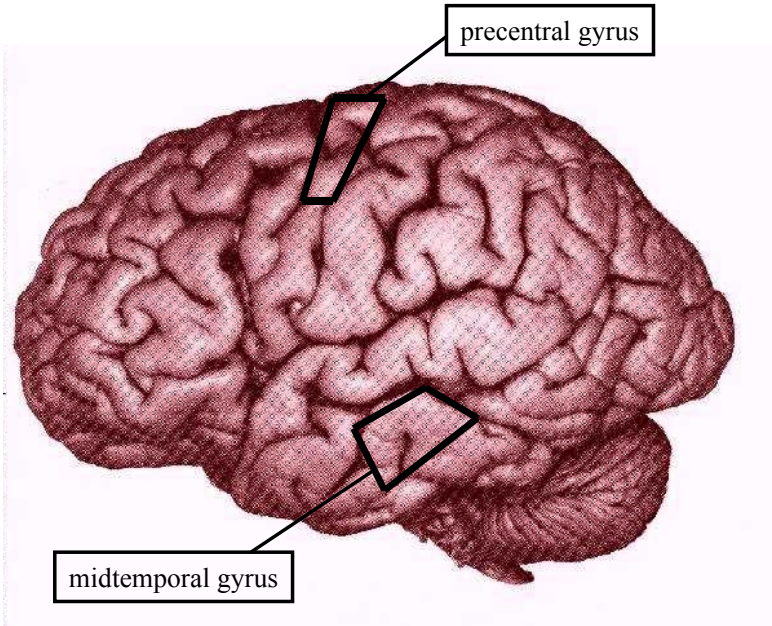



**Fig. 4.** The two brain regions sampled: precentral gyrus and midtemporal gyrus. Precentral gyrus contains the primary motor cortex (Brodmann area 4). Midtemporal gyrus contains the midtemporal association area (Brodmann area 21), which is involved in deductive reasoning [Goel *et al*., 1998] and auditory semantic tasks [Smith *et al*., 1996].

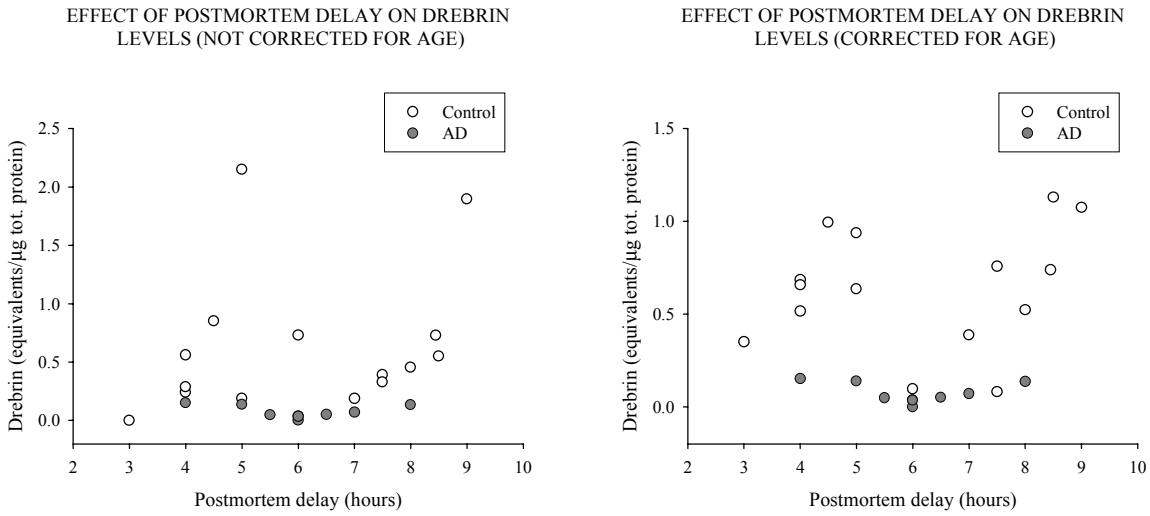



**Fig. 5.** Postmortem interval has no significant effect on drebrin levels within 10 hours of death. Because age had a significant effect on drebrin levels among the controls, absence of correlation between postmortem interval and drebrin levels is shown before (left) and after (right) statistical removal of the effect of age. The effect of age was removed using analysis of covariance.

All Alzheimer tissue was obtained from the brain bank in our laboratory, the Brain Bank of the Laboratory of Neurosciences (National Institute on Aging, Bethesda, MD), which was established during the course of this study. The control group was supplemented with samples obtained from various other brain banks. The diagnoses of Alzheimer's disease were rendered by the Armed Forces Institute of Pathology (AFIP, Bethesda, MD) following published criteria [Mirra *et al*., 1993]. Neuropathological examination of the control brains was also performed and confirmed absence of neurodegenerative diseases. When Alzheimer and control brains were compared in any study, the average age at the time of death was not significantly different between the two groups.

Particular care was paid to rapid freezing of the samples, which was accomplished by immersion in dry ice-cooled isopentane. Care was taken not to let the samples thaw at any point after they had been frozen. All cortical layers were included but white matter and leptomeninges were carefully dissected out.

### 4.2. Immunohistochemistry

The monoclonal PHF-1 antibody for abnormally phosphorylated tau-protein (I-II) as well as the monoclonal M2F6 antibody for drebrin (III) had been previously characterized in published reports [Greenberg *et al*., 1992; Otvos *et al*., 1994; Shirao *et al*., 1989; Shirao *et al*., 1990]. In addition, we demonstrated the specificity of the drebrin antibody by western blotting, where the two isoforms of drebrin, drebrin E and drebrin A [Harigaya *et al*., 1996], were detected as expected at 116 and 125 kD, respectively (Fig. 6).

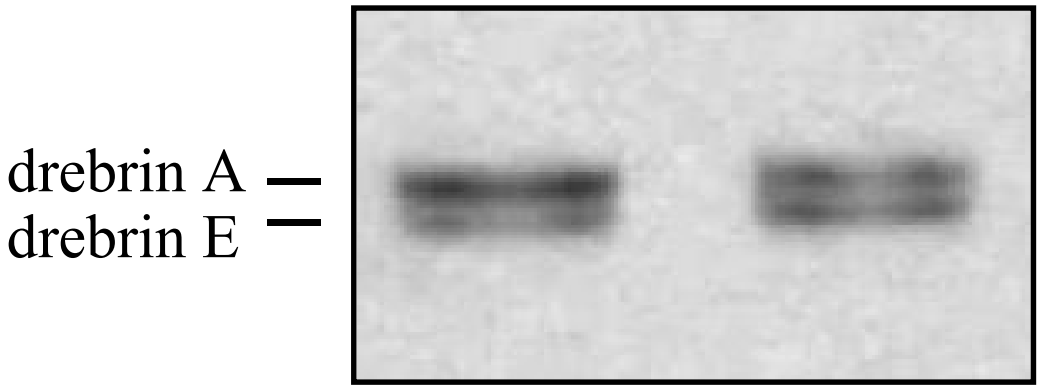



**Fig. 6**. A magnified view of the chemiluminescent signal on a western blot of two human brain samples from Alzheimer midtemporal cortex stained with the monoclonal M2F6 drebrin antibody. The two drebrin isoforms, drebrin A (120 kD) and drebrin E (116 kD), can be distinguished.

Negative controls included omitting the primary antibody, which resulted in no signal. In addition, histological sections from normal control brains and from the relatively unaffected primary motor cortex of Alzheimer brains showed no or very little reactivity to the PHF-1 antibody. The specificity of the PHF-1 antibody was also verified by the characteristic morphology of positive staining of neurofibrillary tangles in neuronal cell bodies and neuronal processes in senile plaques in Alzheimer brains. The immunohistochemical signal for drebrin using the monoclonal M2F6 antibody was undetectable in all samples from Alzheimer midtemporal cortices and in some samples from control midtemporal cortices [Hatanpää *et al*., 1997]. In most samples from control midtemporal cortices, a characteristic punctate staining, known to correspond

to postsynaptic terminals [Shirao, 1995], was obtained [Hatanpää *et al*., 1997]. The specificity of the immunohistochemical staining for drebrin was even easier to verify in sections from the hippocampi of control brains, where individual dendritic spines could be visualized at high magnification (III).

### 4.3. *In situ* hybridization

The $^{35}$S radiolabeled DNA-probes for the mitochondrial DNA encoded human cytochrome oxidase subunit III mRNA and for the mitochondrial DNA encoded 12S ribosomal RNA were prepared polymerase chain reaction from human genomic DNA. The amplified DNA fragments were gel purified, which also confirmed the expected size of the amplified fragments (779 and 782 nucleotides, respectively). The purified fragments of double-stranded DNA were labeled by random primer labeling. Briefly, they were denatured by boiling and incubated with random hexamer primers, Klenow DNA polymerase, dCTP, dGTP, dTTP, and $^{35}$S labeled dATP. Unincorporated dATP and labeled fragments shorter than 20-30 nucleotides were removed using commercial probe purification columns. This so called random primer labeling is known to produce radiolabeled DNA fragments, averaging several hundreds of nucleotides in length, which are complementary to the template DNA. It is widely used to generate probes for northern blotting, but is rarely used for *in situ* hybridization due to concerns of lower sensitivity compared with single-stranded RNA probes and more complicated synthesis compared with oligonucleotide probes. In the present study, however, sensitivity was never a problem, probably because both cytochrome oxidase III mRNA and 12s RNA are relatively highly expressed RNAs.

The specificity of the probes was confirmed by northern blotting, which showed single bands of expected size (II). The specificity of the cytochrome oxidase III mRNA probe was further supported by the observed distribution of the signal at microscopic level, which showed the highest signal in the cell bodies of large pyramidal neurons as well as very low signal in the white matter, which is in agreement with previous reports on the distribution of cytochrome oxidase mRNA in the monkey brain [Chandrasekaran *et*

*al*., 1993]. Negative controls included pretreatment of the histological sections with RNase, which obliterated the signal.

The probe for total mRNA was prepared by end-labeling of a 17-mer oligo(dT) probe using terminal transferase and $^{35}S$ labeled dTTP. This probe is complementary to the oligo(dA) tail present as a stabilizing, noncoding end-sequence in all mRNAs, including mRNAs encoded by the mitochondrial DNA. Therefore, an oligo(dT) probe can be used to obtain a signal corresponding to total mRNA. A negative control probe was prepared from oligo(dA) in an identical way, except that it was labeled with $^{35}S$ dATP. Hybridization using this control probe under identical conditions produced no signal.

### 4.4. Combined *in situ* hybridization and PHF-1 immunohistochemistry

To avoid loss of *in situ* hybridization signal due to RNases present in the blocking serum used in immunohistochemistry, immunohistochemistry using the PHF-1 antibody was performed after *in situ* hybridization. A standard avidin-biotin peroxidase-antiperoxidase protocol was followed and the signal was developed using diaminobenzidine. After immunohistochemistry, the *in situ* hybridization signal was detected using emulsion autoradiography. Negative and positive controls were performed as described above for immunohistochemistry and *in situ* hybridization. In addition, immunohistochemical signal obtained after combined *in situ* hybridization and immunohistochemistry was compared with immunohistochemical signal obtained after immunohistochemistry alone. Although some loss of intensity of the immunohistochemical signal was noted when immunohistochemistry was performed after *in situ* hybridization, there appeared no be no qualitative change in the density and morphology of immunopositive neurofibrillary tangles and senile plaques detected using the PHF-1 antibody.

### 4.5. Quantitative cytochrome oxidase enzyme histochemistry

Standards were used to provide a linear correlation between cytochrome oxidase activity and the optical intensity of staining on sections. Standards were prepared by selecting 6 human brain samples where the range of cytochrome oxidase enzyme activity covered the range of enzyme activity in the experimental samples. Homogenates were prepared from these samples and cytochrome oxidase enzyme activity was determined in the homogenates using a biochemical method (see below). Sections from these standard samples were then prepared and run in parallel with the sections from the experimental samples. The incubation solution was prepared according to a published method [Wong-Riley, 1979]. A variety of different incubation times were tested until an approximately linear relation was achieved between the known cytochrome oxidase enzyme activity in the standards samples and the overall optical staining density (measured through all cortical layers) in sections from the standard samples after cytochrome oxidase enzyme histochemistry. Negative controls included sections pretreated by microwaving and sections incubated in the presence of 0.05 % sodium azide, which is a specific inhibitor of cytochrome oxidase [Bennett *et al*., 1996]. All staining was obliterated in these negative control sections.

### 4.6. Determination of cytochrome oxidase enzyme activity in brain homogenates

Cytochrome oxidase enzyme activity in brain homogenates was performed following a published method [Hevner *et al*., 1993] with minor modifications. The negative control was assay mixture oxidized with potassium ferricyanide.

### 4.7. Quantitative immunoblotting

Quantitative immunoblotting (III) to determine the levels of a number of proteins in brain homogenates was performed using standard dilutions prepared from one of the experimental samples. Brain samples were carefully dissected to obtain full-depth cortical specimens without white matter, homogenized by sonication, and denatured. Equal amounts of total protein from the experimental samples were loaded in a 4 - 20% Tris-Glycine gel (Novex, San Diego, CA). Five to 6 different dilutions of an experimental sample designated to be the standard sample were loaded on each gel; this standard sample was from a 21-year-old control brain. After blotting on nitrocellulose, a reversible staining for total protein (Ponceau reagent, Sigma, St. Louis, MO) was performed to confirm equal loading of the experimental samples. Blots were treated with the primary antibody, followed by a secondary antibody preabsorbed with human serum proteins (KPL, Gaithersburg, MD).

The primary antibodies were (source; apparent size of the detected band; primary and secondary antibody dilutions): synaptophysin mab SVP-38 (Sigma; 38 kD; 1:10,000, 1:2,000), GAP-43 mab GAP-7B10 (Sigma; 46 kD; 1:4,000, 1:2,000), neuron-specific enolase mab (Zymed, South San Francisco, CA; 50 kD; 1:1,500, 1:2,000), βIII-tubulin mab (Promega, Madison, WI; 55 kD; 1:40,000, 1:4,000), and β-tubulin mab (Amersham, Arlington Heights, IL; 55 kD; 1:10,000, 1:2,000).

The chemiluminescent signal was generated using a SuperSignal kit (Pierce, Rockford, IL). The blots were exposed to X-Omat AR 2 film (Kodak, Rochester, NY). Image analysis was performed on optical density-calibrated images captured with a video camera. A “Gel Plotting” macro in NIH Image 1.6 (public domain software written by Wayne Rasband, NIH; available at http://rsb.info.nih.gov/nih-image/download.html) was used to plot the average optical density (O.D.) of each band. The area under each peak, which represents the integrated optical density of the corresponding band, was taken as the signal. Known amounts of protein in the standard dilutions were then plotted against their signal. Curve fitting was performed to obtain an equation to convert the signal of each experimental sample to equivalents of the standard sample

on the same blot. Inclusion of the set of standard dilutions on every blot helped to ensure that quantitative results were obtained and that different blots could be compared. Quantification was performed 3 times with similar results for all proteins, except for neuron-specific enolase and βIII-tubulin, which were measured twice with similar results.

A similar method was used for the quantification of detergent-soluble Aβ, except that dilutions of synthetic Aβ peptide were used as standards (IV). The Aβ-antibody was the 4G8 monoclonal antibody (Senetek PLC, London, UK), which has been previously characterized [Kim *et al*., 1990]. It detects the 17-24 segment of the Aβ–peptide. The signal was detected using the SuperSignal Ultra kit (Pierce).

### 4.8. Statistics

Cytochrome oxidase III mRNA levels in tangle-free and early-tangle bearing neurons (each tangle-free neuron paired with an early-tangle bearing neuron) were compared using a two-way ANOVA for repeated measures (I) [AbacusConcepts, 1989]. Tangle-free, early-tangle bearing, and late-tangle bearing neurons (grouped in triplets) were compared using a two-way Student-Newmann-Keuls multiple comparisons test (I) [AbacusConcepts, 1989].

Tangle-free neurons in Alzheimer's disease and control cases were compared using an ANOVA for unequally nested designs [Neter *et al*., 1985]. Because randomly selected pyramidal neurons in the Alzheimer's disease samples tended to be slightly smaller than neurons in the control samples, the effect of neuronal size was removed from the grain counts by analysis of covariance [Keppel, 1982].

A two-tailed t-test was used to compare average cytochrome oxidase III mRNA and poly(A)$^+$ levels in neurons close (< 20 μm) and far (> 60 μm) from plaques (II). Because the lack of significant differences could have resulted from "noise" in the data introduced by different levels of mRNA in different Alzheimer brains and even

different levels of mRNA in neurons around different plaques in the same brain, analysis of covariance was used to remove the effects of these factors. However, the differences remained statistically nonsignificant.

Linear regression analysis was used to test the significance of correlation between age and levels of the various proteins quantified in brain samples (III, IV). As it could be argued that these relations are not necessarily linear, the significance of correlation was also tested for nonlinear correlation using the Kendall's rank correlation and Spearman's rank correlation tests, but the results remained essentially the same. Significance of difference in average levels of the various proteins between Alzheimer and control groups was tested using a two-tailed t-test. All significant differences would have remained significant also after adjustment for multiple comparisons within each group of proteins (plasticity-related, structural, and energy metabolic proteins) using the Bonferroni multiple comparisons test [Shaffer, 1988].

## 5. MAJOR RESULTS

In Alzheimer's disease midtemporal cortex, cytochrome oxidase III mRNA decreased in individual neurons as the formation of neurofibrillary tangles progressed. Comparing Alzheimer's disease tangle-free neurons with control tangle-free neurons, Alzheimer's disease tangle-free neurons had lower levels of cytochrome oxidase III mRNA. 12S rRNA, also encoded by mitochondrial DNA, and poly $(A)^+$ mRNA, representing total mRNA, were not decreased in Alzheimer's disease tangle-free neurons and were decreased to a lesser extent than cytochrome oxidase III mRNA during neurofibrillary tangle formation. (I)

Proximity of neurons to senile plaques was not associated with decreased cytochrome oxidase III mRNA or poly $(A)^+$ mRNA levels in Alzheimer's disease midtemporal cortex. Cytochrome oxidase enzyme activity in neuronal processes close to senile plaques also was not decreased. Overall cytochrome oxidase III mRNA levels were decreased in Alzheimer's disease midtemporal cortex compared with controls, but this decrease did not correlate with senile plaque density. (II)

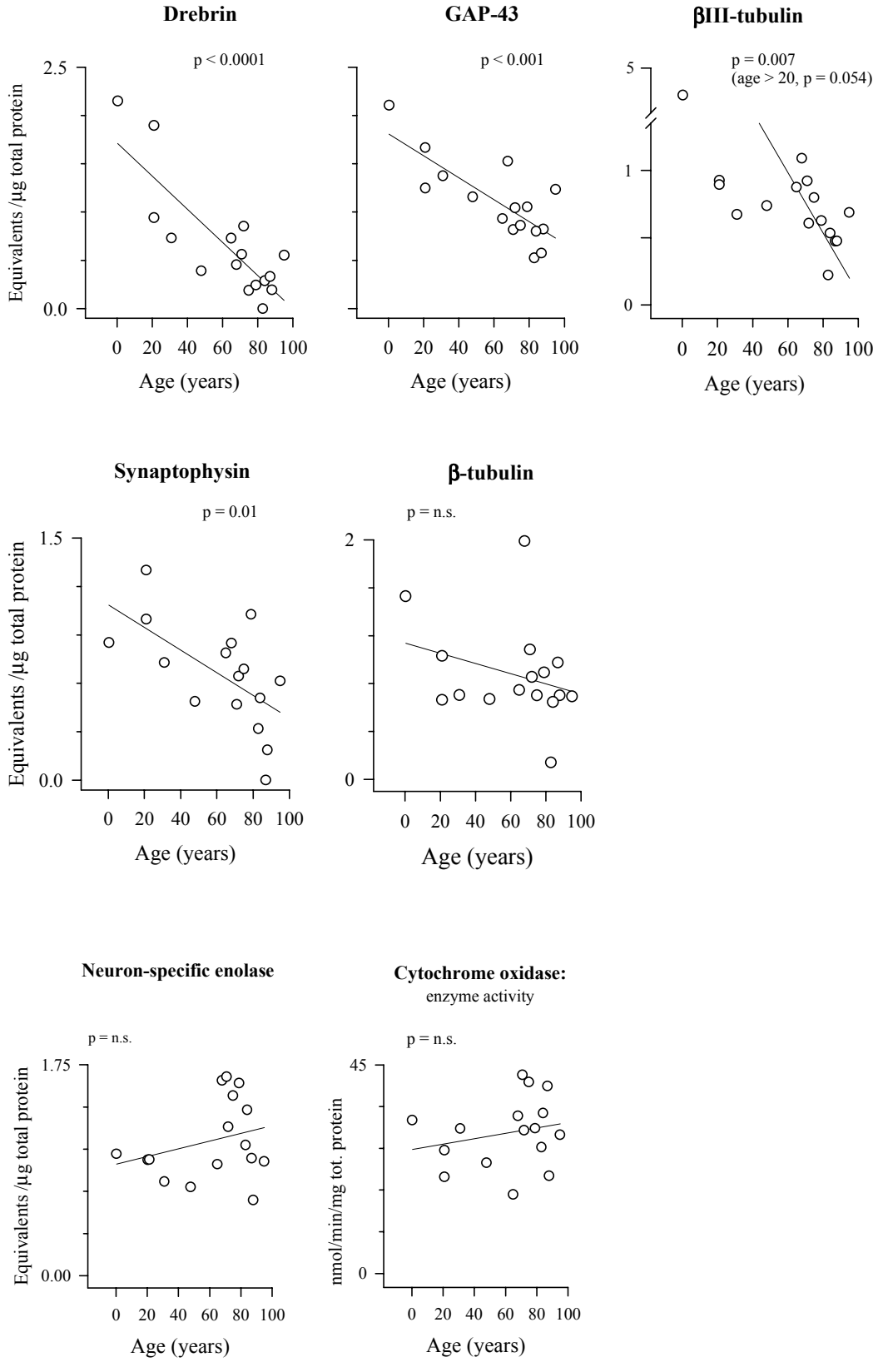
Cytochrome oxidase enzyme activity, a marker of oxidative energy metabolism, and levels of neuron specific enolase, a marker of glycolytic energy metabolism, remained unchanged in normal aging. Of the two energy metabolic markers, cytochrome oxidase enzyme activity was 49% lower in Alzheimer's disease midtemporal cortex compared with elderly controls ($p = 0.0003$), whereas neuron specific enolase levels were not significantly different in the two groups. During normal aging, there was a marked decline in levels of drebrin ($p = 0.0001$), GAP-43 ($p < 0.001$), and βIII-tubulin ($p = 0.007$), which are proteins regulating synaptic structural plasticity, in midtemporal cortex. (III, Fig. 7)

Of the 2 mainly structural proteins, synaptophysin also showed a small but significant decline in normal aging ($p = 0.01$), whereas β-tubulin remained unchanged. In Alzheimer brains, drebrin levels were decreased by 81% compared with elderly

controls ($p = 0.002$). A smaller but statistically significant decrease was seen in βIII-tubulin levels ($p = 0.04$), whereas only a nonsignificant trend ($p = 0.055$) for lower levels was found in synaptophysin levels. (III)

Compared with controls, levels of detergent-soluble Aβ were markedly elevated in Alzheimer's disease midtemporal cortex. In Alzheimer brains, levels of detergent-soluble Aβ levels were substantially higher than previously reported for water-soluble Aβ. Detergent-soluble Aβ levels did not correlate significantly with cytochrome oxidase enzyme activity or levels of drebrin in Alzheimer's disease midtemporal cortex. (IV)

**Fig. 7**. Effect of age on levels of plasticity markers (drebrin, GAP-43, βIII tubulin), primarily structural proteins (synaptophysin, β tubulin), and energy metabolic markers (cytochrome oxidase enzyme activity, neuron-specific enolase) in the human brain (midtemporal cortex). The most significant decreases are seen in levels of the plasticity markers. These graphs are identical to those presented in figures 2C, D, and E of III, except that one additional sample from a 4-month-old infant has been added.


Drebrin
p < 0.0001
Equivalents /µg total protein
2.5
0.0
0 20 40 60 80 100
Age (years)
GAP-43
p < 0.001
0 20 40 60 80 100
Age (years)
βIII-tubulin
p = 0.007
(age > 20, p = 0.054)
5
1
0
0 20 40 60 80 100
Age (years)
Synaptophysin
p = 0.01
Equivalents /µg total protein
1.5
0.0
0 20 40 60 80 100
Age (years)
β-tubulin
p = n.s.
2
0
0 20 40 60 80 100
Age (years)
Neuron-specific enolase
p = n.s.
Equivalents /µg total protein
1.75
0.00
0 20 40 60 80 100
Age (years)
Cytochrome oxidase:
enzyme activity
p = n.s.
nmol/min/mg tot. protein
45
0
0 20 40 60 80 100
Age (years)

# 6. DISCUSSION

## 6.1. Methodological considerations

### 6.1.1. Labeling of *in situ* hybridization probes

Probes for *in situ* hybridization were labeled by random primer labeling. This method produces radiolabeled DNA fragments, averaging several hundreds of nucleotides in length, which are complementary to the template DNA. It is widely used to generate probes for northern blotting, but is rarely used for *in situ* hybridization due to concerns of lower sensitivity compared with single-stranded RNA probes and more complicated synthesis compared with oligonucleotide probes. In the present study, however, sensitivity was never a problem, probably because both cytochrome oxidase III mRNA and 12s RNA are relatively highly expressed RNAs.

### 6.1.2. Quantitative cytochrome oxidase enzyme histochemistry

Enzyme histochemistry is a method which allows localization and quantification of the enzyme activity of a particular enzyme in histological sections. Specific enzyme histochemical staining methods for dozens of enzymes were developed and widely used until the 1970's, when advances in immunohistochemistry and antibody generation made immunohistochemistry a more popular and fashionable method for the histological detection of enzymes.

However, there are a number of important applications where enzyme histochemistry still has its place. For example, cytochrome oxidase enzyme histochemistry (Fig. 8) is a rapid, inexpensive, and highly specific method for the detection of cytochrome oxidase enzyme activity [Wong-Riley, 1989a]. More importantly, quantitative results can be obtained by the use enzyme activity standards [Gonzalez-Lima and Garrosa, 1991].

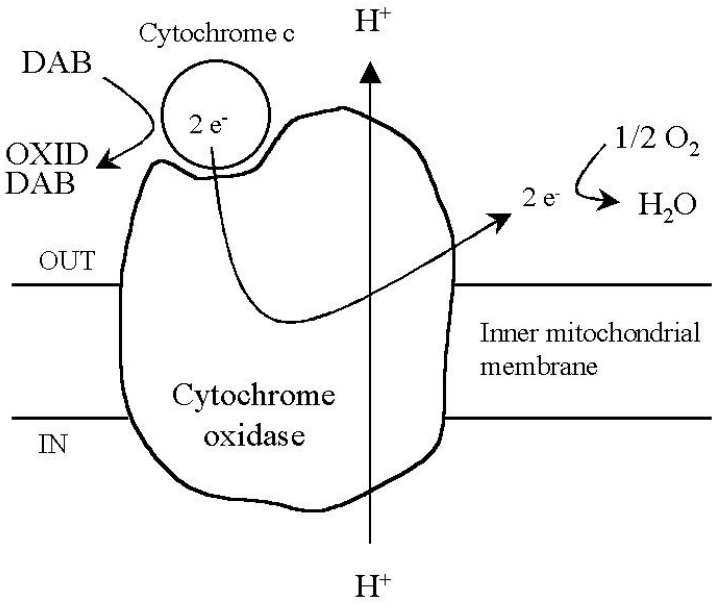



**Fig. 8.** Principle of cytochrome oxidase enzyme histochemistry. Cytochrome oxidase (complex IV of the respiratory chain) catalyses the transfer of electrons from cytochrome c to oxygen, forming water. In living tissue, the electron donor is complex III (cytochrome reductase). For each electron transferred, one proton is used to form water and one proton is pumped across the inner mitochondrial membrane. The proton gradient is used by the $F_1F_0$ ATP synthase to synthesize ATP. In the histochemical reaction, as shown in this figure, the electron donor is diaminobenzidine (DAB). The reduced form of DAB is colorless and soluble, whereas oxidized DAB forms a brown precipitate, allowing its microscopic localization at the site of enzyme activity. In the biochemical method, where cytochrome oxidase enzyme activity is measured in tissue homogenates, reduced cytochrome c is added to the homogenates. The rate of decrease in absorbance at 550 nm, which reflects oxidation of cytochrome c, is then measured using a spectrophotometer. Modified from [Wong-Riley, 1989a] and [Gennis and Ferguson-Miller, 1995].

In the present study, quantitative cytochrome oxidase enzyme histochemistry was used to determine if senile plaques have any direct effects on energy metabolism in neuronal processes (II). The answer to this question cannot be obtained by the use of *in situ* hybridization to cytochrome oxidase mRNA, because cytochrome oxidase mRNA is mainly present in neuronal cell bodies rather than in neuronal processes [Hevner and Wong-Riley, 1991].

### 6.1.3. Determination of cytochrome oxidase enzyme activity in brain homogenates

Cytochrome oxidase enzyme activity in brain homogenates was performed following a published method [Hevner *et al*., 1993] with minor modifications. A number of other methods has been published [Kish *et al*., 1992; Nobrega *et al*., 1993; Wharton and Tzagoloff, 1967]; the underlying basic principle of all these methods is making use of the fact that the absorption maximum of reduced cytochrome c is different from that of oxidized cytochrome c. Hence, it is possible to measure cytochrome oxidase enzyme activity in an aliquot of a tissue homogenate by placing it in a spectrophotometer, adding reduced cytochrome c and monitoring the decrease in absorbance at the absorption maximum of reduced cytochrome c (550 nm). Using the known molar extinction coefficient difference for reduced cytochrome c, cytochrome oxidase enzyme activity can then be calculated from the change in absorbance per minute and expressed as nanomoles of cytochrome c oxidized per microgram of total protein in the homogenate.

### 6.1.4. Determination of Aβ levels in brain homogenates by quantitative immunoblotting

The sensitivity of the present method in detecting Aβ (8 pg/lane) compares favorably with most previously published methods. For example, one recent study reported a detection limit of 20 pg/lane for detecting Aβ42 using a western blotting method and 12 pmol/g wet weight using an enzyme immunoassay (EIA) method [Funato *et al*., 1998]

## 6.2. Neuronal energy metabolism in aging

We found no significant change in cytochrome oxidase enzyme activity in midtemporal association neocortex during aging (III). These results are in line with reports of no change or a slight increase in cytochrome oxidase enzyme activity and

mRNA in the cerebral cortex of the rat [Gorini *et al*., 1989; Nicoletti *et al*., 1998; Villa and Gorini, 1991]. Our findings are surprising in light of the decline in glucose uptake during aging in the human brain [Grady *et al*., 1998; Petit-Taboue *et al*., 1998]. One possible explanation for this apparent discrepancy is that cytochrome oxidase is a marker of oxidative phosphorylation, not glucose uptake or glycolysis. Therefore, it is possible that glucose uptake or the rate of glycolysis is decreased in aging even though the rate of oxidative phosphorylation is not. In support of this explanation, it has been reported that levels of several, although not all, glycolytic enzymes decrease in the rat brain during aging [Leong *et al*., 1981]. Our finding of no change in neuron specific enolase levels in aging (III) is not necessarily in conflict with the idea of decreased glycolysis in aging. Neuron specific enolase, being a neuronal protein, may reflect the rate of neuronal glycolysis rather than total glycolysis in the brain. A major part of glycolysis in the brain occurs in astrocytes, which take up glucose and process it to lactate. Lactate secreted by astrocytes is then taken up by neurons, which convert lactate to pyruvate for use in oxidative phosphorylation [Tsacopoulos and Magistretti, 1996]. Therefore, it is possible that the reported decline in cerebral glucose uptake during aging reflects a decline in glucose uptake or glycolysis in astrocytes rather than decreased oxidative phosphorylation in neurons.

### 6.3. Synaptic plasticity in aging and Alzheimer's disease

Levels of drebrin in the midtemporal association neocortex were significantly depressed in normal aging and even more so in AD patients (81% lower than elderly controls). In the adult brain, drebrin is localized mainly in dendritic spines, which are postsynaptic terminals of excitatory synapses. In view of the findings that dendritic spine density is markedly decreased during normal aging of the human brain and that additional spines are lost in AD [Catala *et al*., 1988; Jacobs *et al*., 1997], the present observations of decreased drebrin levels in aging and AD may reflect loss of dendritic spines. In AD, instability of phospholipid membranes [Ginsberg *et al*., 1995] may interfere with maintenance of spines, leading to disassembly of their microfilament scaffold and the associated proteins, including drebrin. On the other hand, because

drebrin maintains the form of dendritic spines by regulating the microfilament scaffold [Hayashi *et al*., 1996; Shirao, 1995], loss of drebrin in aging and AD may promote spine dysfunction and instability, leading to loss of spines. In any case, loss or dysfunction of dendritic spines may seriously diminish the ability of neurons to modulate synaptic input and to isolate toxic concentrations of intracellular calcium [Segal, 1995].

### 6.4. Pathologic basis of neuronal dysfunction in Alzheimer's disease

Recently, Kish et al., who were the first to demonstrate that cytochrome oxidase enzyme activity is decreased in Alzheimer brains [Kish *et al*., 1992], extended their original study by measuring levels of cytochrome oxidase subunits in temporal and parietal cortices in brains of patients with Alzheimer's disease, spinocerebellar ataxia type I, and Friedreich's ataxia, as well as in normal controls [Kish *et al*., 1999]. The two hereditary ataxias, where decreased brain energy metabolism has been reported by positron emission tomography, were included to assess disease specificity of the alterations in cytochrome oxidase levels. Cytochrome oxidase subunit levels were found to be decreased in both Alzheimer's disease and in the two hereditary ataxias. The authors cite our work and conclude that the decrease in cytochrome oxidase levels is not specific to Alzheimer's disease, but likely occurs secondary to neurodegeneration, possibly reflecting downregulation in response to reduced energy demand.

A similar view was presented by Bonilla et al. [Bonilla *et al*., 1999], who refer to our studies in their recent review paper on mitochondrial involvement in Alzheimer's disease. They concur with our interpretation that there is presently no convincing evidence to support the notion that decreased cerebral energy metabolism in Alzheimer's disease is caused by mutations and deletions in mitochondrial DNA. Like us, they argue that decreased energy metabolism in Alzheimer's disease most likely results from physiological downregulation [Chandrasekaran *et al*., 1996;

Chandrasekaran *et al*., 1998; Chandrasekaran *et al*., 1997; Fukuyama *et al*., 1996; Hatanpää *et al*., 1996; Hatanpää *et al*., 1998; Rapoport *et al*., 1996].

The association of a progressive decrease in cytochrome oxidase mRNA level with the intraneuronal accumulation neurofibrillary tangles in Alzheimer's disease suggests that neurofibrillary tangles contribute to decreased cerebral energy metabolism in Alzheimer's disease (I). However, these results also suggest that even neurons bearing neurofibrillary tangles continue to function, albeit at a lower level. It is therefore possible that neurons in the early stages of tangle development are not irreversibly lost but might be still responsive to appropriate therapy.

The observation that not only neurons bearing tangles but also tangle-free neurons show a decrease in cytochrome oxidase mRNA suggests that tangle formation may not be the primary cause of neurodegeneration in Alzheimer's disease. As animal experiments have shown a tight coupling between the expression of cytochrome oxidase and synaptic firing, synaptic dysfunction or degeneration may precede tangle formation and possible be the primary site from which the degeneration spreads to the neuronal cell body, ultimately leading to the death of the neuron. While the mechanism of this degenerative process is not known, one possibility is that that aging-related impairments in structural plasticity of the neuronal cytoskeleton or aging-related loss of phospholipid membrane stability [Ginsberg *et al*., 1995] interfere with the ability of dendritic spines to isolate the massive influx of calcium to the postsynaptic terminal, which is associated with synaptic firing. In normal aging, loss of synapses resulting from impaired capacity to isolate calcium in the postsynaptic terminal is minimal and not sufficient to cause dementia. However, in some individuals, who will later develop Alzheimer's disease, the extent of synapse loss may reach a threshold level, leading to downregulation of mitochondrial energy metabolism. Under normal circumstances, this downregulation would be a physiological response to conserve energy when there is no need to spend it. However, downregulated mitochondrial energy metabolism also renders neurons more vulnerable to sudden energy demand, such as the sudden energy demand that is associated with calcium influx. Hence, the stage is set for a vicious circle: synapse loss leads to downregulation of energy metabolism, which leads to

vulnerability to more synapse loss and neurodegeneration. Ultimately, neurodegeneration progresses to a point where clinical dementia becomes evident.

Although the hypothesis outlined above is admittedly speculative, it is consistent with the available evidence. For example, it has been shown that inhibition of mitochondrial energy metabolism leads to greater vulnerability of neurons to toxicity of excitatory neurotransmitters (excitotoxicity) [Ludolph *et al*., 1993]. Also, our finding that proteins regulating synaptic structural plasticity, such as drebrin, are disproportionately lost during normal aging suggests that impairment of structural plasticity is an important feature of normal aging (III). Therefore, the hypothesis outlined above would explain why Alzheimer's disease is so prominently associated with old age and never affects young adults.

How does Aβ peptide fit into this hypothesis? One possibility is that Aβ enhances excitotoxicity, like it has been shown to do both *in vitro* and in vivo [Mattson *et al*., 1992; Morimoto *et al*., 1998]. In any case, either Aβ peptide itself or abnormal processing of the amyloid precursor protein is clearly implicated in the pathogenesis of Alzheimer's disease. As mentioned in the introduction, accumulation of Aβ peptide in the extracellular space as plaques is an important diagnostic feature of both sporadic and familial Alzheimer's disease. In addition, all familial Alzheimer's disease-associated mutations identified so far affect the processing of amyloid precursor protein in such a way that production of the long forms of Aβ peptide (Aβ42/43) is increased [Mann *et al*., 1996; Price *et al*., 1998b]. On the other hand, the role of senile plaques, which are extracellular aggregations of Aβ fibrils, has been questioned as the cause of Alzheimer's disease because of weak or absent correlations between plaque numbers and dementia severity and between plaque numbers and neuronal loss. Also, our own results demonstrated the lack of association between plaques and decreased cytochrome oxidase mRNA and enzyme activity in Alzheimer brains (II). This suggests that senile plaques, unlike neurofibrillary tangles (I), do not contribute to decreased energy metabolism in Alzheimer's disease, and therefore likely are not a part of the pathologic cascade leading to Alzheimer's disease.

However, Aβ is present in Alzheimer brains not only in fibrillar form in senile plaques, but also in a nonfibrillar, more soluble form, which is the precursor of Aβ in plaques and at least in part intracellular. Neurotoxicity of nonfibrillar Aβ has been demonstrated in cell culture studies. Therefore, our attention was turned to nonfibrillar Aβ. We measured the levels of nonfibrillar (detergent-soluble) Aβ in Alzheimer's disease and control midtemporal brain homogenates and compared these values with the levels of drebrin and cytochrome oxidase enzyme activity in the same samples (IV). No significant correlations were found, suggesting that nonfibrillar Aβ is also not responsible for the decreased neuronal energy metabolism in Alzheimer's disease.

There are still a number of ways not addressed by our studies as to how Aβ or abnormal processing of amyloid precursor protein could cause Alzheimer's disease. For example, Aβ could be toxic only when present in a particular intracellular compartment or when bound to a certain intracellular protein, thus explaining why levels of total Aβ correlate poorly with neuronal injury. There is some experimental evidence to support this argument. For example, according to one study, *in vitro* neurotoxicity of Aβ is dependent on its binding to an intracellular polypeptide known as ERAB, which is expressed in normal tissues, but is overexpressed in neurons affected in Alzheimer's disease [Yan *et al*., 1997]. The toxic effect of Aβ on cultured cells is prevented by blocking ERAB and is enhanced by overexpression of ERAB [Yan *et al*., 1997]. According to another line of research, the toxic peptide released by abnormal processing of amyloid precursor protein may not be Aβ, but a longer fragment derived from the carboxy terminal of the amyloid precursor protein [Kim *et al*., 1999a; Kim *et al*., 1999b; Kim *et al*., 1998; Song *et al*., 1998]. Interestingly, this longer peptide may be neurotoxic due to its ability to form $Ca^{2+}$ permeable cation channels on neuronal membranes [Kim *et al*., 1999a].

# 7. CONCLUSIONS

These results, combined with other available evidence, suggest that:

- Neurofibrillary tangles, but not senile plaques, contribute to decreased cerebral energy metabolism in Alzheimer's disease.

- Neurofibrillary tangles, senile plaques, and elevated brain levels of total nonfibrillar Aβ - although prominent features of Alzheimer's disease - are probably not the primary causes of neurodegeneration.

- Disturbed mechanisms of structural synaptic plasticity may contribute to cognitive dysfunction in normal aging and Alzheimer's disease.

## ACKNOWLEDGEMENTS

This study was carried out during the years 1993-1998 in the Laboratory of Neurosciences (Chief: Stanley I. Rapoport, M.D.), National Institute on Aging, Bethesda, Maryland, the United States. The primary source of financial support was the intramural program of the National Institute on Aging. In addition, I received grants from the Finnish Cultural Foundation and the University of Tampere.

I wish to express my deepest gratitude to the principal supervisor of this thesis, Professor **Antti Hervonen**, M.D., Ph.D., Laboratory of Gerontology, Department of Public Health, University of Tampere Medical School. It was in his laboratory at the University of Tampere Medical School where I learned the basic scientific skills that formed the groundwork for the present study. Without his encouragement and support this thesis would never have materialized. I am greatly indebted to **Stanley I. Rapoport**, M.D., Ph.D. (hon.), Chief of the Laboratory of Neurosciences and co-supervisor of this thesis, for his invaluable scientific guidance and for providing the excellent facilities and training opportunities which made this study possible.

My warmest thanks are due to my co-author and mentor at the Laboratory of Neurosciences, **Krish "Chandra" Chandrasekaran**, Ph.D. His dedication and friendship have been a source of great inspiration to me. While at the Laboratory of Neurosciences, I was very fortunate to have the opportunity to train with **Daniel R. Brady**, Ph.D., an expert neuroanatomist. I wish to express my sincere thanks for all his help and advice during the course of this study.

I am indebted to my co-authors **James Stoll**, Ph.D., **Zygmunt Galdzicki**, Ph.D., and **Li-Ing Liu**, Ph.D., who worked with me at the Laboratory of Neurosciences. It was a great pleasure to get to know and to collaborate with **Tomoaki Shirao**, M.D., Ph.D., Gunma University School of Medicine, Japan, **Bakshy A. Chibber**, Ph.D., University of Notre Dame, and **Krystyna R. Isaacs**, Ph.D., National Institute of Mental Health. Conversations with **A. David Purdon**, Ph.D., **Ruyichi "Lou" Fukuyama**, M.D.,

Ph.D., **Umesha Shetty**, Ph.D., **Sheryl K. Brining**, Ph.D., **M. Catherine Bennett**, Ph.D., and **Larisa D. Acevedo**, Ph.D., at the Laboratory of Neurosciences were helpful and are gratefully acknowledged. I am indebted to **Jyrki Rintala**, M.D., Ph.D., for his help in obtaining additional clinical information on the subjects.

I am thankful to **Jari Koistinaho**, M.D., Ph.D. for his advice and for creating an inspiring work environment while I was a member of the research group at the Laboratory of Gerontology, University of Tampere Medical School. I want to extend my warm thanks to the colleagues I had the privilege to befriend there, who included **Sirkka Lahtivirta**, M.D., Ph.D., **Jaana Suhonen**, M.D., Ph.D., and **Reina Roivainen**, M.D., Ph.D., as well as to the entire gerontology group. I am grateful to **Timo Koivula**, M.D., Ph.D., chairman of the Department of Clinical Chemistry, University Hospital of Tampere, for his encouragement and support.

The prompt and careful review of this work by **Jouni Sirviö**, M.D., Ph.D., is gratefully appreciated.

My sincere thanks are due to Ms. **Ruth Seemann**, Ms. **Evelyn Dangerfield**, Ms. **Ritva Määttä**, Ms. **Oili Kemppainen**, and Ms. **Ulla Jukarainen** for their skillful technical assistance. I want to thank **Drew Olsen**, M.D., and **Robert Camp**, M.D., Ph.D., my chief residents, and **José Costa**, M.D., chairman of the Anatomic Pathology Department at Yale, for allowing me to use my elective time to finish this thesis.

My heartfelt thanks go to my parents Mrs. **Leena Hatanpää** and Mr. **Kalevi Hatanpää** for always emphasizing the importance of hard work and education and for encouraging me to continue my studies as far as I wanted to go. Finally, I would like to thank my dear wife **Krystyna R. Isaacs**, Ph.D., for her love and unwavering confidence in me.

New Haven, November 7, 1999

Kimmo Hatanpää

## REFERENCES


AbacusConcepts. SuperANOVA. Berkeley, CA: Abacus Concepts, Inc., 1989.

Allen DD, Galdzicki Z, Brining SK, Fukuyama R, Rapoport SI, and Smith QR. Beta-amyloid induced increase in choline flux across PC12 cell membranes. Neurosci Lett 1997; 234: 71-3.

Alzheimer A. About a peculiar disease of the cerebral cortex. (Translated by L. Jarvik and H. Greenson) [classical article]. Alzheimer Dis Assoc Disord 1987; 1: 3-8.

Alzheimer A. On certain peculiar diseases of old age. History of Psychiatry 1991; 2: 74-101.

Arendt T, Bruckner MK, Bigl V, and Marcova L. Dendritic reorganisation in the basal forebrain under degenerative conditions and its defects in Alzheimer's disease. II. Ageing, Korsakoff's disease, Parkinson's disease, and Alzheimer's disease. J Comp Neurol 1995; 351: 189-222.

Asada H, Uyemura K, and Shirao T. Actin-binding protein, drebrin, accumulates in submembranous regions in parallel with neuronal differentiation. J Neurosci Res 1994; 38: 149-59.

Bennett MC, Mlady GW, Kwon YH, and Rose GM. Chronic in vivo sodium azide infusion induces selective and stable inhibition of cytochrome c oxidase. J Neurochem 1996; 66: 2606-11.

Berchtold NC, and Cotman CW. Evolution in the conceptualization of dementia and Alzheimer's disease: Greco-Roman period to the 1960s. Neurobiol Aging 1998; 19: 173-89.

Berent S, Giordani B, Foster N, Minoshima S, Lajiness-O'Neill R, Koeppe R, and Kuhl DE. Neuropsychological function and cerebral glucose utilization in isolated memory impairment and Alzheimer's disease. J Psychiatr Res 1999; 33: 7-16.

Blacker D, Wilcox MA, Laird NM, Rodes L, Horvath SM, Go RC, Perry R, Watson B, Jr., Bassett SS, McInnis MG, Albert MS, Hyman BT, and Tanzi RE. Alpha-2 macroglobulin is genetically associated with Alzheimer disease [see comments]. Nat Genet 1998; 19: 357-60.

Blass JP. Age-associated memory impairment and Alzheimer's disease [editorial; comment]. J Am Geriatr Soc 1996; 44: 209-11.

Bonilla E, Tanji K, Hirano M, Vu TH, DiMauro S, and Schon EA. Mitochondrial involvement in Alzheimer's disease. Biochim Biophys Acta 1999; 1410: 171-82.

Borowsky I, and Collins R. Histochemical changes in enzymes of energy metabolism in the dentate gyrus accompany deafferentation and synaptic reorganization. Neuroscience 1989a; 33: 253-262.

Borowsky I, and Collins R. Metabolic anatomy of brain: a comparison of regional capillary density, glucose metabolism, and enzyme activities. J Comp Neurol 1989b; 288: 401-413.

Braak H, and Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol Aging 1997; 18: 351-7.

Carroll EW, and Wong-Riley MT. Quantitative light and electron microscopic analysis of cytochrome oxidase-rich zones in the striate cortex of the squirrel monkey. J Comp Neurol 1984; 222: 1-17.

Catala I, Ferrer I, Galofre E, and Fabregues I. Decreased numbers of dendritic spines on cortical pyramidal neurons in dementia. A quantitative Golgi study on biopsy samples. Hum Neurobiol 1988; 6: 255-9.

Chandrasekaran K, Stoll J, Rapoport SI, and Brady DR. Localization of cytochrome oxidase (COX) activity and COX mRNA in the perirhinal and superior temporal sulci of the monkey brain. Brain Res 1993; 606: 213-9.

Chandrasekaran K, Giordano T, Brady D, Stoll J, Martin L, and Rapoport S. Impairment in mitochondrial cytochrome oxidase gene expression in Alzheimer disease. Mol Brain Res 1994; 24: 336-340.

Chandrasekaran K, Hatanpää K, Brady D, and Rapoport S. Evidence for physiological downregulation of brain oxidative phosphorylation in Alzheimer's disease. Exp Neurol 1996; 142: 80-88.

Chandrasekaran K, Hatanpää K, Rapoport SI, and Brady DR. Decreased expression of nuclear and mitochondrial DNA-encoded genes of oxidative phosphorylation in association neocortex in Alzheimer disease. Brain Res Mol Brain Res 1997; 44: 99-104.

Chandrasekaran K, Hatanpää K, Brady DR, Stoll J, and Rapoport SI. Downregulation of oxidative phosphorylation in Alzheimer disease: loss of cytochrome oxidase

subunit mRNA in the hippocampus and entorhinal cortex. Brain Res 1998; 796: 13-9.

Christie G, Markwell RE, Gray CW, Smith L, Godfrey F, Mansfield F, Wadsworth H, King R, McLaughlin M, Cooper DG, Ward RV, Howlett DR, Hartmann T, Lichtenthaler SF, Beyreuther K, Underwood J, Gribble SK, Cappai R, Masters CL, Tamaoka A, Gardner RL, Rivett AJ, Karran EH, and Allsop D. Alzheimer's disease: correlation of the suppression of beta-amyloid peptide secretion from cultured cells with inhibition of the chymotrypsin-like activity of the proteasome. J Neurochem 1999; 73: 195-204.

Cook DG, Forman MS, Sung JC, Leight S, Kolson DL, Iwatsubo T, Lee VM, and Doms RW. Alzheimer's A beta(1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. Nat Med 1997; 3: 1021-3.

Crook T, and Larrabee GJ. Age-associated memory impairment: diagnostic criteria and treatment strategies. Psychopharmacol Bull 1988; 24: 509-14.

Crook TH, and Ferris SH. Age associated memory impairment [letter; comment] [see comments]. BMJ 1992; 304: 714.

Crook R, Ellis R, Shanks M, Thal LJ, Perez-Tur J, Baker M, Hutton M, Haltia T, Hardy J, and Galasko D. Early-onset Alzheimer's disease with a presenilin-1 mutation at the site corresponding to the Volga German presenilin-2 mutation. Ann Neurol 1997; 42: 124-8.

Crook R, Verkkoniemi A, Perez-Tur J, Mehta N, Baker M, Houlden H, Farrer M, Hutton M, Lincoln S, Hardy J, Gwinn K, Somer M, Paetau A, Kalimo H, Ylikoski R, Poyhonen M, Kucera S, and Haltia M. A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1 [see comments]. Nat Med 1998; 4: 452-5.

Cummings BJ, Pike CJ, Shankle R, and Cotman CW. Beta-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease [see comments]. Neurobiol Aging 1996; 17: 921-33.

Davis JN, 2nd, and Chisholm JC. Alois Alzheimer and the amyloid debate [letter; comment]. Nature 1999; 400: 810.

DeKosky ST, and Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 1990; 27: 457-64.

DeKosky ST, Scheff SW, and Styren SD. Structural correlates of cognition in dementia: quantification and assessment of synapse change. Neurodegeneration 1996; 5: 417-21.

Delaere P, Duyckaerts C, Masters C, Beyreuther K, Piette F, and Hauw JJ. Large amounts of neocortical beta A4 deposits without neuritic plaques nor tangles in a psychometrically assessed, non-demented person. Neurosci Lett 1990; 116: 87-93.

Deyoe E, Trusk T, and Wong-Riley M. Activity correlates of cytochrome oxidase-defined compartments in granular and supragranular layers of primary visual cortex of the macaque monkey. Vis Neurosci 1995; 12: 629-639.

Dickson DW, Crystal HA, Mattiace LA, Masur DM, Blau AD, Davies P, Yen SH, and Aronson MK. Identification of normal and pathological aging in prospectively studied nondemented elderly humans. Neurobiol Aging 1992; 13: 179-89.

Difiglia M, Graveland GA, and Schiff L. Cytochrome oxidase activity in the rat caudate nucleus: light and electron microscopic observations. J Comp Neurol 1987; 255: 137-45.

Ehrenstein G, Galdzicki Z, and Lange GD. The choline-leakage hypothesis for the loss of acetylcholine in Alzheimer's disease. Biophys J 1997; 73: 1276-80.

Fabiani M, and Friedman D. Dissociations between memory for temporal order and recognition memory in aging. Neuropsychologia 1997; 35: 129-41.

Flicker C, Ferris SH, and Reisberg B. Mild cognitive impairment in the elderly: predictors of dementia. Neurology 1991; 41: 1006-9.

Fujii M, Katayama Y, Makiyama Y, Maejima S, and Tsubokawa T. Dynamic changes in cytochrome oxidase activity in the rat somatosensory cortex following thalamocortical deafferentation . Neurol Res 1993; 15: 384-388.

Fukuyama R, Hatanpää K, Rapoport SI, and Chandrasekaran K. Gene expression of ND4, a subunit of complex I of oxidative phosphorylation in mitochondria, is decreased in temporal cortex of brains of Alzheimer's disease patients. Brain Res 1996; 713: 290-3.

Funato H, Yoshimura M, Kusui K, Tamaoka A, Ishikawa K, Ohkoshi N, Namekata K, Okeda R, and Ihara Y. Quantitation of amyloid beta-protein (A beta) in the cortex during aging and in Alzheimer's disease. Am J Pathol 1998; 152: 1633-40.

Galdzicki Z, Fukuyama R, Wadhwani K, Rapoport S, and Ehrenstein G. Beta-Amyloid increases choline conductance of PC12 cells: possible mechanism of toxicity in Alzheimer's disease. Brain Res 1994; 646: 332-336.

Gennis R, and Ferguson-Miller S. Structure of cytochrome c oxidase, energy generator of aerobic life [comment]. Science 1995; 269: 1063-4.

Ginsberg L, Rafique S, Xuereb J, Rapoport S, and Gershfeld N. Disease and anatomic specificity of ethanolamine plasmalogen deficiency in Alzheimer's disease brain. Brain Res 1995; 698: 223-226.

Goel V, Gold B, Kapur S, and Houle S. Neuroanatomical correlates of human reasoning. J Cogn Neurosci 1998; 10: 293-302.

Gonzalez-Lima F, and Garrosa M. Quantitative histochemistry of cytochrome oxidase in rat brain. Neurosci Lett 1991; 123: 251-3.

Gorini A, Arnaboldi R, Ghigini B, and Villa RF. Brain cytochrome oxidase activity of synaptic and nonsynaptic mitochondria during aging. Basic Appl Histochem 1989; 33: 139-45.

Grady CL, McIntosh AR, Bookstein F, Horwitz B, Rapoport SI, and Haxby JV. Age-related changes in regional cerebral blood flow during working memory for faces. Neuroimage 1998; 8: 409-25.

Greenberg S, Davies P, Schein J, and Binder L. Hydrofluoric acid-treated tau-PHF proteins display the same biochemical properties as normal tau. J Biol Chem 1992; 267: 564-569.

Haltia M, Viitanen M, Sulkava R, Ala-Hurula V, Poyhonen M, Goldfarb L, Brown P, Levy E, Houlden H, Crook R, and et al. Chromosome 14-encoded Alzheimer's disease: genetic and clinicopathological description [see comments]. Ann Neurol 1994; 36: 362-7.

Hardy J. The Alzheimer family of diseases: many etiologies, one pathogenesis? [comment]. Proc Natl Acad Sci U S A 1997; 94: 2095-7.

Harigaya Y, Shoji M, Shirao T, and Hirai S. Disappearance of actin-binding protein, drebrin, from hippocampal synapses in Alzheimer's disease. J Neurosci Res 1996; 43: 87-92.

Hartmann T, Bieger SC, Bruhl B, Tienari PJ, Ida N, Allsop D, Roberts GW, Masters CL, Dotti CG, Unsicker K, and Beyreuther K. Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. Nat Med 1997; 3: 1016-20.

Hatanpää K, Brady DR, Stoll J, Rapoport SI, and Chandrasekaran K. Neuronal activity and early neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1996; 40: 411-20.

Hatanpää K, Shirao T, Brady DR, Chandrasekaran K, and Rapoport SI. Immunoreactivity for drebrin, a marker of dendritic plasticity, in human cerebral cortex in normal aging and Alzheimer's disease. Soc Neurosci Abstr 1997; 23: 1650.

Hatanpää K, Chandrasekaran K, Brady DR, and Rapoport SI. No association between Alzheimer plaques and decreased levels of cytochrome oxidase subunit mRNA, a marker of neuronal energy metabolism. Brain Res Mol Brain Res 1998; 59: 13-21.

Hatanpää K, Isaacs KR, Shirao T, Brady DR, and Rapoport SI. Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. J Neuropathol Exp Neurol 1999; 58: 637-43.

Hayashi K, Ishikawa R, Ye LH, He XL, Takata K, Kohama K, and Shirao T. Modulatory role of drebrin on the cytoskeleton within dendritic spines in the rat cerebral cortex. J Neurosci 1996; 16: 7161-70.

Hayashi K, and Shirao T. Change in the shape of dendritic spines caused by overexpression of drebrin in cultured cortical neurons. J Neurosci 1999; 19: 3918-25.

Heinonen O, Soininen H, Sorvari H, Kosunen O, Paljarvi L, Koivisto E, and Riekkinen PJ, Sr. Loss of synaptophysin-like immunoreactivity in the hippocampal formation is an early phenomenon in Alzheimer's disease. Neuroscience 1995; 64: 375-84.

Hevner RF, and Wong-Riley MT. Neuronal expression of nuclear and mitochondrial genes for cytochrome oxidase (CO) subunits analyzed by in situ hybridization: comparison with CO activity and protein. J Neurosci 1991; 11: 1942-58.

Hevner R, and Wong-Riley M. Mitochondrial and nuclear gene expression for cytochrome oxidase subunits are disproportionally regulated by functional activity in neurons. J Neurosci 1993; 13: 1805-1819.

Hevner RF, Liu S, and Wong-Riley MT. An optimized method for determining cytochrome oxidase activity in brain tissue homogenates. J Neurosci Methods 1993; 50: 309-19.

Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, and Duff K. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med 1998; 4: 97-100.

Howlett DR, Perry AE, Godfrey F, Swatton JE, Jennings KH, Spitzfaden C, Wadsworth H, Wood SJ, and Markwell RE. Inhibition of fibril formation in beta-amyloid peptide by a novel series of benzofurans. Biochem J 1999; 340: 283-9.

Hyman B, Irizarry M, Soriano F, McNamara M, Page K, Schenk D, and Games D. Aβ deposition is associated with neuritic changes and gliosis, but not with overt neuronal loss in the PDAPP transgenic mouse. Soc Neurosci Abstr 1997; 23: 1115.

Ibanez V, Pietrini P, Alexander GE, Furey ML, Teichberg D, Rajapakse JC, Rapoport SI, Schapiro MB, and Horwitz B. Regional glucose metabolic abnormalities are not the result of atrophy in Alzheimer's disease. Neurology 1998; 50: 1585-93.

Irizarry MC, McNamara M, Fedorchak K, Hsiao K, and Hyman BT. APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1 [see comments]. J Neuropathol Exp Neurol 1997a; 56: 965-73.

Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, and Hyman BT. Abeta deposition is associated with neuropil changes, but not with overt

neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J Neurosci 1997b; 17: 7053-9.

Islam K, and Levy E. Carboxyl-terminal fragments of beta-amyloid precursor protein bind to microtubules and the associated protein tau. Am J Pathol 1997; 151: 265-71.

Jacobs B, Driscoll L, and Schall M. Life-span dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi study. J Comp Neurol 1997; 386: 661-80.

Jellinger KA, and Bancher C. Neuropathology of Alzheimer's disease: a critical update. J Neural Transm Suppl 1998; 54: 77-95.

Jones EG, Hendry SH, and Brandon C. Cytochrome oxidase staining reveals functional organization of monkey somatosensory thalamus. Exp Brain Res 1986; 62: 438-42.

Kageyama GH, and Wong-Riley MT. Histochemical localization of cytochrome oxidase in the hippocampus: correlation with specific neuronal types and afferent pathways. Neuroscience 1982; 7: 2337-61.

Keppel G. Design and analysis. Englewood Cliffs, NJ: Prentice-Hall, Inc., 1982.

Kim KS, Wen GY, C. B, Chen CMJ, Sapienza VJ, Hong H, and Wisniewski HM. Detection and quantitation of amyloid b-peptide with 2 monoclonal antibodies. Neuroscience Research Communications 1990; 7: 113-121.

Kim HS, Park CH, and Suh YH. C-terminal fragment of amyloid precursor protein inhibits calcium uptake into rat brain microsomes by Mg2+-Ca2+ ATPase. Neuroreport 1998; 9: 3875-9.

Kim HJ, Suh YH, Lee MH, and Ryu PD. Cation selective channels formed by a C-terminal fragment of beta- amyloid precursor protein [In Process Citation]. Neuroreport 1999a; 10: 1427-31.

Kim HS, Lee JH, and Suh YH. C-terminal fragment of Alzheimer's amyloid precursor protein inhibits sodium/calcium exchanger activity in SK-N-SH cell. Neuroreport 1999b; 10: 113-6.

Kish S, Bergeron C, and Rajput A. Brain cytochrome oxidase in Alzheimer's disease. J Neurochem 1992; 59: 776-779.

Kish SJ, Mastrogiacomo F, Guttman M, Furukawa Y, Taanman JW, Dozic S, Pandolfo M, Lamarche J, DiStefano L, and Chang LJ. Decreased brain protein levels of cytochrome oxidase subunits in Alzheimer's disease and in hereditary spinocerebellar ataxia disorders: a nonspecific change? J Neurochem 1999; 72: 700-7.

Kuhl DE, Koeppe RA, Minoshima S, Snyder SE, Ficaro EP, Foster NL, Frey KA, and Kilbourn MR. In vivo mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease [see comments]. Neurology 1999; 52: 691-9.

LaFerla FM, Troncoso JC, Strickland DK, Kawas CH, and Jay G. Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Abeta stabilization. J Clin Invest 1997; 100: 310-20.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, and Klein WL. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A 1998; 95: 6448-53.

Leong SF, Lai JC, Lim L, and Clark JB. Energy-metabolizing enzymes in brain regions of adult and aging rats. J Neurochem 1981; 37: 1548-56.

Ludolph AC, Riepe M, and Ullrich K. Excitotoxicity, energy metabolism and neurodegeneration. J Inherit Metab Dis 1993; 16: 716-23.

Mann DM, Iwatsubo T, Cairns NJ, Lantos PL, Nochlin D, Sumi SM, Bird TD, Poorkaj P, Hardy J, Hutton M, Prihar G, Crook R, Rossor MN, and Haltia M. Amyloid beta protein (Abeta) deposition in chromosome 14-linked Alzheimer's disease: predominance of Abeta42(43). Ann Neurol 1996; 40: 149-56.

Mattson M, Cheng B, Davis D, Bryant K, Lieberburg I, and Rydel R. Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci 1992; 12: 376-398.

McPhie DL, Lee RKK, Eckman CB, Olstein DH, Durham SP, Yager D, Younkin SG, Wurtman RJ, and Neve RL. Neuronal expression of beta-amyloid precursor protein Alzheimer mutations causes intracellular accumulation of a C-terminal fragment containing both the amyloid beta and cytoplasmic domains. J Biol Chem 1997; 272: 24743-6.

Mielke R, Kessler J, Szelies B, Herholz K, Wienhard K, and Heiss WD. Normal and pathological aging--findings of positron-emission-tomography. J Neural Transm 1998; 105: 821-37.

Mirra S, Hart M, and Terry R. Making the diagnosis of Alzheimer's disease. Arch Pathol Lab Med 1993; 117: 132-144.

Moller HJ, and Graeber MB. The case described by Alois Alzheimer in 1911. Historical and conceptual perspectives based on the clinical record and neurohistological sections. Eur Arch Psychiatry Clin Neurosci 1998; 248: 111-22.

Morimoto K, Yoshimi K, Tonohiro T, Yamada N, Oda T, and Kaneko I. Co-injection of beta-amyloid with ibotenic acid induces synergistic loss of rat hippocampal neurons. Neuroscience 1998; 84: 479-87.

Morris JC, McKeel DW, Jr., Storandt M, Rubin EH, Price JL, Grant EA, Ball MJ, and Berg L. Very mild Alzheimer's disease: informant-based clinical, psychometric, and pathologic distinction from normal aging [see comments]. Neurology 1991; 41: 469-78.

Morris JC, Storandt M, McKeel DW, Jr., Rubin EH, Price JL, Grant EA, and Berg L. Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. Neurology 1996; 46: 707-19.

Morrison JH, and Hof PR. Life and death of neurons in the aging brain. Science 1997; 278: 412-9.

Mufson EJ, Chen EY, Cochran EJ, Beckett LA, Bennett DA, and Kordower JH. Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. Exp Neurol 1999; 158: 469-90.

Mutisya E, Bowling A, and Beal M. Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. J. Neurochem. 1994; 63: 2179-2184.

Neter J, Wasserman W, and Kutner MH. Applied Linear Statistical Models. Homewood, Illinois: Richard D. Irwin, Inc., 1985.

Neve R. Mixed signals in Alzheimer's disease. TINS 1996; 19: 371-372.

Nicoletti VG, Tendi EA, Console A, Privitera A, Villa RF, Ragusa N, and Giuffrida-Stella AM. Regulation of cytochrome c oxidase and FoF1-ATPase subunits expression in rat brain during aging. Neurochem Res 1998; 23: 55-61.

Nobrega JN, Raymond R, DiStefano L, and Burnham WM. Long-term changes in regional brain cytochrome oxidase activity induced by electroconvulsive treatment in rats. Brain Res 1993; 605: 1-8.

Nomura S, and Mizuno N. Histochemical demonstration of vibrissae-representing patchy patterns of cytochrome oxidase activity within the trigeminal sensory nuclei in the cat. Brain Res 1986; 380: 167-71.

Otvos L, Feiner L, and Lang E. Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. J Neurosci Res 1994; 39: 669-673.

Perez-Tur J, Croxton R, Wright K, Phillips H, Zehr C, Crook R, Hutton M, Hardy J, Karran E, Roberts GW, Lancaster S, and Haltia T. A further presenilin 1 mutation in the exon 8 cluster in familial Alzheimer's disease. Neurodegeneration 1996; 5: 207-12.

Petit-Taboue MC, Landeau B, Desson JF, Desgranges B, and Baron JC. Effects of healthy aging on the regional cerebral metabolic rate of glucose assessed with statistical parametric mapping. Neuroimage 1998; 7: 176-84.

Pietrini P, Furey ML, Alexander GE, Mentis MJ, Dani A, Guazzelli M, Rapoport SI, and Schapiro MB. Association between brain functional failure and dementia severity in Alzheimer's disease: resting versus stimulation PET study. Am J Psychiatry 1999; 156: 470-3.

Price DL, Sisodia SS, and Borchelt DR. Alzheimer disease--when and why? [news; comment]. Nat Genet 1998a; 19: 314-6.

Price DL, Sisodia SS, and Borchelt DR. Genetic neurodegenerative diseases: the human illness and transgenic models. Science 1998b; 282: 1079-83.

Rapoport S. Positron emission tomography in Alzheimer's disease in relation to disease pathogenesis: a critical review. Cerebrovasc Brain Metab Rev 1991; 3: 297-335.

Rapoport S, Hatanpää K, Brady D, and Chandrasekaran K. Brain energy metabolism, cognitive function and downregulated oxidative phosphorylation in Alzheimer ddisease. Neurodegeration 1996; 5: 473-476.

Riekkinen P, Jr., Schmidt BH, and van der Staay FJ. Animal models in the development of symptomatic and preventive drug therapies for Alzheimer's disease. Ann Med 1998; 30: 566-76.

Rosenblum WI. The presence, origin, and significance of A beta peptide in the cell bodies of neurons. J Neuropathol Exp Neurol 1999; 58: 575-81.

Schaie KW. The hazards of cognitive aging. Gerontologist 1989; 29: 484-93.

Schaie KW. The course of adult intellectual development. Am Psychol 1994; 49: 304-13.

Scheff SW, DeKosky ST, and Price DA. Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol Aging 1990; 11: 29-37.

Scheff SW, and Price DA. Synapse loss in the temporal lobe in Alzheimer's disease. Ann Neurol 1993; 33: 190-9.

Scheff SW, Price DA, Hamrick J, C.T. C, and Sparks DL. Estimation of possible age-related changes in synaptic density in the human frontal cortex (Brodman area 9). Soc Neurosci Abstr 1997; 231: 650.

Segal M. Dendritic spines for neuroprotection: a hypothesis. Trends Neurosci 1995; 18: 468-71.

Selkoe DJ, Abraham CR, Podlisny MB, and Duffy LK. Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem 1986; 46: 1820-34.

Selkoe DJ. Alzheimer's disease: a central role for amyloid [see comments]. J Neuropathol Exp Neurol 1994; 53: 438-47.

Shaffer JP. Simultaneous testing. In: Kotz S and Johnson NL, editors. Encyclopedia of Statistical Sciences. New York: John Wiley & Sons, 1988: 484-490.

Sherratt EJ, Thomas AW, and Alcolado JC. Mitochondrial DNA defects: a widening clinical spectrum of disorders [editorial]. Clin Sci (Colch) 1997; 92: 225-35.

Shirao T, and Obata K. Two acidic proteins associated with brain development in chick embryo. J Neurochem 1985; 44: 1210-6.

Shirao T, and Obata K. Immunochemical homology of 3 developmentally regulated brain proteins and their developmental change in neuronal distribution. Brain Res 1986; 394: 233-44.

Shirao T, Kojima N, Nabeta Y, and Obata K. Two forms of drebrin, developmentally regulated brain proteins, in rat. Proc Jpn Acad 1989; 65: 169-172.

Shirao T, Kojima N, Terada S, and Obata K. Expression of three drebrin isoforms in the developing nervous system. Neurosci Res Suppl 1990; 13: S106-11.

Shirao T, and Obata K. [The role of neuronal cytoskeleton associated proteins in neuronal network formation]. Nippon Yakurigaku Zasshi 1992; 100: 465-73.

Shirao T. The roles of microfilament-associated proteins, drebrins, in brain morphogenesis: a review. J Biochem (Tokyo) 1995; 117: 231-6.

Simonian N, and Hyman B. Functional alterations in Alzheimer's disease: diminution of cytochrome oxidase in the hippocampal formation. J Neuropathol Exp Neurol 1994a; 52: 580-585.

Simonian N, and Hyman B. Functional alterations in Alzheimer's disease: selective loss of mitochondrial-encoded cytochrome oxidase mRNA in the hippocampal formation. J Neuropathol Exp Neurol 1994b; 53: 508-512.

Smith CD, Andersen AH, Chen Q, Blonder LX, Kirsch JE, and Avison MJ. Cortical activation in confrontation naming. Neuroreport 1996; 7: 781-5.

Song DK, Won MH, Jung JS, Lee JC, Kang TC, Suh HW, Huh SO, Paek SH, Kim YH, Kim SH, and Suh YH. Behavioral and neuropathologic changes induced by central injection of carboxyl-terminal fragment of beta-amyloid precursor protein in mice. J Neurochem 1998; 71: 875-8.

Suh YH. An etiological role of amyloidogenic carboxyl-terminal fragments of the beta-amyloid precursor protein in Alzheimer's disease. J Neurochem 1997; 68: 1781-91.

Svennerholm L, and Gottfries CG. Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early-onset form (type I) and demyelination in late-onset form (type II). J Neurochem 1994; 62: 1039-47.

Swaab DF, Lucassen PJ, Salehi A, Scherder EJ, van Someren EJ, and Verwer RW. Reduced neuronal activity and reactivation in Alzheimer's disease. Prog Brain Res 1998; 117: 343-77.

Toda M, Shirao T, Minoshima S, Shimizu N, Toya S, and Uyemura K. Molecular cloning of cDNA encoding human drebrin E and chromosomal mapping of its gene. Biochem Biophys Res Commun 1993; 196: 468-72.

Toda M, Shirao T, and Uyemura K. Suppression of an actin-binding protein, drebrin, by antisense transfection attenuates neurite outgrowth in neuroblastoma B104 cells. Brain Res Dev Brain Res 1999; 114: 193-200.

Tootell RB, Silverman MS, Hamilton SL, De Valois RL, and Switkes E. Functional anatomy of macaque striate cortex. III. Color. J Neurosci 1988; 8: 1569-93.

Troncoso JC, Sukhov RR, Kawas CH, and Koliatsos VE. In situ labeling of dying cortical neurons in normal aging and in Alzheimer's disease: correlations with senile plaques and disease progression. J Neuropathol Exp Neurol 1996; 55: 1134-42.

Tsacopoulos M, and Magistretti PJ. Metabolic coupling between glia and neurons. J Neurosci 1996; 16: 877-85.

Unger JM, van Belle G, and Heyman A. Cross-sectional versus longitudinal estimates of cognitive change in nondemented older people: a CERAD study. Consortium to Establish a Registry for Alzheimer's Disease. J Am Geriatr Soc 1999; 47: 559-63.

Vasan S, Zhang X, Zhang X, Kapurniotu A, Bernhagen J, Teichberg S, Basgen J, Wagle D, Shih D, Terlecky I, Bucala R, Cerami A, Egan J, and Ulrich P. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo. Nature 1996; 382: 275-278.

Villa RF, and Gorini A. Action of L-acetylcarnitine on different cerebral mitochondrial populations from hippocampus and striatum during aging. Neurochem Res 1991; 16: 1125-32.

Wharton D, and Tzagoloff A. Cytochrome oxidase from beef mitochondria. Methods Enzymol 1967; 10: 245-250.

Wisniewski T, Ghiso J, and Frangione B. Biology of A beta amyloid in Alzheimer's disease [published erratum appears in Neurobiol Dis 1998 Jul;5(1):65]. Neurobiol Dis 1997; 4: 313-28.

Wong-Riley MT. Endogenous perioxidatic activity in brain stem neurons as demonstrated by their staining with diaminobenzidine in normal squirrel monkeys. Brain Res 1976; 108: 257-77.

Wong-Riley MT, Merzenich MM, and Leake PA. Changes in endogenous enzymatic reactivity to DAB induced by neuronal inactivity. Brain Res 1978; 141: 185-92.

Wong-Riley M. Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res 1979; 171: 11-28.

Wong-Riley M, Walsh S, Leake-Jones P, and Merzenich M. Maintenance of neuronal activity by electrical stimulation of unilaterally deafened cats demonstrable with cytochrome oxidase technique. Ann Otol Rhinol Laryngol Suppl 1981; 90: 30-32.

Wong-Riley M. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci. 1989a; 12: 94-101.

Wong-Riley MT. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci 1989b; 12: 94-101.

Wood S, MacKenzie L, Maleeff B, Hurle M, and Wetzel R. Selective inhibition of Abeta fibril formation. J Biol Chem 1996; 271: 4086-4092.

Yan SD, Fu J, Soto C, Chen X, Zhu H, Al-Mohanna F, Collison K, Zhu A, Stern E, Saido T, Tohyama M, Ogawa S, Roher A, and Stern D. An intracellular protein that binds amyloid-beta peptide and mediates neurotoxicity in Alzheimer's disease [see comments]. Nature 1997; 389: 689-95.

## ORIGINAL PUBLICATIONS I-IV