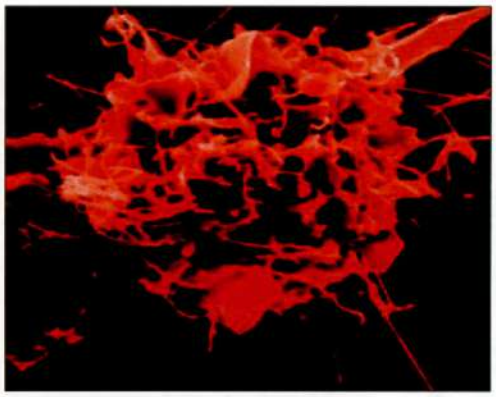

Editorial office:

"Ana Aslan" International Academy of Aging®
"Ana Aslan" International Foundation®
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ISSN 1582-8352 (printed)
ISSN 1582-8360 (on-line)

Brain Aging International Journal®
Ana Aslan International Academy of Aging®

The accepted abbreviation for **Brain Aging International Journal** for bibliographic citation is **Brain Aging**

EDITORIAL

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CASE REPORT

Scientific board

Hypertension and Lifestyle Modifications in the Elderly

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Physiological effects of aging include reduced myocardial contractility, reduced glomerular filtration, reduced hepatic blood flow and mass, reduced cognitive functions, reduced splanhnic blood flow, total body water, total lean body mass, and increased body fat. Thus, the volume of distribution of water-soluble drugs may be smaller in the elderly, and the lipid-soluble drugs may have a longer elimination time. Theoretically, these can favor the higher likelihood of adverse reactions in older persons.

The prevalence of hypertension increases with aging. According to the National Health and Nutrition Examination Survey III, 1988-1991, the distribution for the population 60 years and older was 49.6% for stage 1 hypertension (140-159/90-99 mmHg) and 24.7% for stage 2 hypertension (>160/100 mmHg). The prevalence for women exceeds that of men between the fifth and sixth decade of life. The control rate of hypertension is defined as systolic blood pressure less than 140 mmHg and diastolic blood pressure less than 90 mmHg. It is especially important that blood pressure control be achieved in older patients to avoid the debilitating complications of stroke, heart failure, and renal failure.

A study from 1993 has documented that systolic blood pressure is a better predictor of cardiovascular events than diastolic blood pressure. This observation confirmed the original data from the Framingham Study that identified systolic blood pressure as a prime risk factor for cardiovascular diseases and focused again attention on the treatment of systolic blood pressure, especially in elderly patients.

The high prevalence of hypertension in older persons suggests that the recognition and treatment of hypertension in elderly should be a priority for physicians. Awareness and treatment for hypertension have been increased in older patients, but despite this improvement, hypertension continues to be poorly controlled in this patient population. Complex drug regimens can result in noncompliance, and adverse drug reactions are increased if high doses of a single drug are used. The American Society of Geriatric Cardiology together with the Joint National Committee of High Blood Pressure emphasize that lifestyle modification should be a part of the treatment of hypertension, regardless of the patient`s age. The recommended lifestyle modifications are especially weight loss, dietary changes with a diet low in fat and protein, increased physical activity and limiting of alcohol intake, sodium intake

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and smoking. It is often said that elderly patients do not change their lifestyle. But if they follow this lifestyle therapy, most of the trials, including our own research, have documented benefit from blood pressure lowering.

The impact of diet in the general population is significant. Despite the smaller effect of dietary factors on blood pressure, there are significant changes that can be achieved. Since dietary habits are potentially modifiable, the manipulation of diet could have a significant effect on blood pressure levels, but also in the rise in blood pressure with age.

Combination drug therapy may often be necessary to provide additional efficacy in elderly patients. Since most of the data trials show that the overall response rate is low, it may be necessary that more elderly patients should receive advices of lifestyle modification and drug therapy combination.

In summary, the treatment of hypertension in elderly patients requires considerations about the possible adverse drug reactions, but it is important that blood pressure control be achieved to avoid the debilitating effects of complications that impair the quality of life of older patients.

Structural and Functional Correlates in the Aging of the Cerebral Cortex

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Abstract

In recent years, we have investigated the impact of aging on the structural and functional parameters of the rat cerebral cortex. Our studies have included behavioural assessments, light and electron microscopic investigations, and the electrophysiological analyses of individual, fully characterized, lamina V cortical neurons in tissue slices, which were intracellularly filled with markers to allow a detailed analysis of their morphology and synaptic patterns. These investigations revealed that large pyramidal neurons are vulnerable to the aging process, during which they experience an important reduction in cell body size as well as loss of dendritic processes, in particular distal branches of basilar dendrites, accompanied by significant synaptic loss. This loss is greatest in the cholinergic system, which preferentially establishes synapses on the shaft of pyramidal neuronal dendrites. Our electrophysiological analysis revealed a loss of functional synaptic connections to pyramidal neurons, yet a compensatory increase in synaptic drive in the aged brain. Moreover, in aged rats displaying cognitive impairment, we found a marked decrease in the number of cholinergic pre-synaptic boutons in the cerebral cortex. We also found that the delivery of a small, preoteolically stable agonist of the TrkA receptor, called D3, was capable of reversing the spatial impairment in these aged impaired rats, leading to a long-lasting rescue of cholinergic pre-synaptic boutons to levels comparable to young and aged unimpaired animals. This small molecule with selective activity may have a considerable therapeutic potential.

Keywords: *cholinergic, pyramidal neurons, cortical synapses, trophic factors, aged-related cognitive impairment, dendritic atrophy.*

Introduction

There is compelling scientific evidence that the human aging process is accompanied by changes in the cerebral cortex leading to the deterioration of higher CNS functions. It is assumed that these changes are at the heart of the well-known cognitive deficiencies observed in old age. Similar changes have been observed in experimental animals. These changes include the disappearance of dendrites, dendritic spines and synapses. Since the number of neurons appears to remain stable in the aged cerebral cortex, the loss of both pre- and post-synaptic structures is thought to have the most impact on the maintenance of inter-neuronal connections. Most of the available data points towards an age-dependent loss of pre-synaptic elements without defining the neurotransmitter involved. Neurochemical studies, nevertheless, would suggest a significant involvement of cholinergic mechanisms in the aging process[1-5]. This notion is reinforced by the elegant demonstration that age-related cognitive impairments correlate with cellular atrophy of forebrain cholinergic neurons[6-9]. A great deal of attention has been paid to the effect of aging on these cholinergic cell bodies, while relatively little attention has focused on their termination sites in the cerebral cortex and the hippocampus, where ultimately the neurotransmitter acetylcholine (ACh) acts on target neurons. In this

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review we summarize some of our efforts to attempt to correlate age-related structural cortical synaptic alterations with functional changes.

Neuronal loss

The most striking feature of the aging human brain is their shrinkage[10-12]. The advent of magnetic resonance imaging has provided accurate non-invasive proof of brain shrinkage with age, most notably of cortical mass[13,14].

Extensive neuronal loss has long been suggested to be the primary factor explaining age-related brain shrinkage. A number of studies have reported that cell loss in the aged neocortex is primarily due to loss of large neurons[10,15,16], although loss of small neurons has also been reported[17,18]. However, the occurrence of extensive neuronal loss in aged brains has been questioned by Haug and coworkers[19], suggesting that the number of neurons in young brains has been overestimated. After correcting for shrinkage, Haug and Eggers[20] observed not a loss but an *increase* in neuronal density accompany by a decreased brain volume in the aged cerebral cortex. An independent study by Terry et al[16] also supported no age-related cell loss in the cerebral cortex. After careful analysis of brains from patients who had died of neurodegenerative diseases, Terry et al[16] showed that large neurons in aged brains decreased significantly in number but that the small neurons increased to an equal extent. This finding suggests that a good number of the large neurons may shrink with age, thus explaining the apparent increase in small neurons in the aged brain.

Others investigators have found no age-related loss of cortical neurons in both monkeys[21] and humans[22]. It has been reported that neuronal numbers in the cerebral cortex of rats remain unchanged with age[21,23]. In line with observations in the human brain, we have recently demonstrated a very prominent shrinkage of the large pyramidal neurons in the rat[24].

In summary, there is no conclusive evidence supporting a significant loss of neurons with age. Instead, the shrinkage of the numerous large pyramidal neurons and the loss of white matter could be important factors contributing to the significant age-related brain shrinkage in humans. In addition, Alzheimer's disease (AD) cell shrinkage is probably more marked in discrete neuronal populations, such as cholinergic neurons of the nucleus basalis of Meynert[25].

Dendritic loss in aging

About 90% of the total receptive surface area of cortical neurons is constituted by dendrites[26,27]. Presynaptic boutons establish synaptic contacts on dendritic shafts and on specialized dendritic structures, namely the dendritic spines. Most synapses containing excitatory neurotransmitters, like glutamate, establish contacts on dendritic spines[28,29].

In the following lines we will summarize current ideas on age-related dendritic changes in cerebral cortex pyramidal and nonpyramidal neurons. Pyramidal neurons are the major projection neurons of the cerebral cortex[29,30], are present in all cortical layers except layer I, and constitute approximately 70-80% of the total neuronal population in the cerebral cortex.

Although pyramidal neurons display some heterogeneity, typical pyramidal neurons carry the following three structural characteristics: (1) a typical dendritic tree, with a prominent apical dendrite directed radially towards the brain surface, from which oblique dendrites arise, and basal dendrites that originate from the cell body and extend laterally or downward; (2) all dendrites possess abundant dendritic spines; and (3) an axon extending from the cell body directed downwards towards other cortical or subcortical regions and which has local axonal collaterals. Apart from these typical pyramidal neurons, there are examples of pyramidal neurons not possessing some of the above-mentioned morphologies.

Since pyramidal neurons represent the most significant cortical neuronal populations, we have focused on structural/ physiological alterations of these neurons during the aging process. Significant age-related loss of dendrites in pyramidal neurons in the human cerebral cortex was first reported by Scheibel and coworkers[31,32]. Among different dendritic compartments of pyramidal neurons, age-related modifications appear to be earliest and most marked in basal dendrites. Significant loss and shortening of basal dendrites has been known for some time now[31,33,34]. In addition to the shortening of dendritic structures, basal dendrites in aged brains of diverse species also have fewer branches[33,35-38]. Apart from a major loss of basal dendrites, age-related losses of oblique and apical dendrites have also been reported in the aged brain[35,39]. It is important to note that dendritic losses in aged brains are not inevitable. For instance, no loss of dendrites in layer II pyramidal neurons has been reported in the entorhinal cortex of aged rats[40].

Loss of dendritic spines, in particular in basal dendrites, is also a characteristic change of aged pyramidal neurons[33,34,37,41,42]. Taken together, these studies provide evidence for a substantial diminution of the dendritic surface of pyramidal neurons in aged brains. Since dendrites are the major receptive membrane for synaptic inputs[26,43], the marked loss of dendritic structures should limit the postsynaptic substrate available for synaptic connections in the aged cerebral cortex. We have reported significant changes in a number of morphological parameters of lamina V pyramidal in the parietal cortex of aged rats. With respect to the total number of basal dendrites branches, pyramidal neurons from aged animals also pos-

sessed significantly fewer branches than those from young rats[24]. Consequently, the total length of basal dendrites was also significantly decreased in aged rats. We also found a significant age-related decrease in the number of high-order distal basal dendritic branches. Parallel to the loss of basal dendrites, the density of spines on these dendrites was significantly lower in aged rats[24].

Synaptic changes

Numerous studies of synaptic loss during normal aging have been performed in the last two decades.

Quantitative studies using electron microscopy revealed significant loss of synapses with age in laboratory animals[44,45] and humans[46,47]. However, not every kind of synapse is altered equally with age. Adams's group has reported age-related loss of asymmetrical synapses, but not symmetrical synapses, in the layer I region of the somatosensory cortex in aged humans[46]. Similar preferential loss of asymmetric synapses was also observed in the hippocampus[48]. Alternatively, synapses on dendritic spines (mostly asymmetric) are more likely to be affected during aging when compared with synapses on dendritic shafts[44,49].

Apart from synaptic loss, age-related modification of synaptic structure has been reported. Adams and Jones[44] showed that terminals in the parietal cortex of aged rats contain fewer mitochondria, synaptic vesicles, reduced vacuolar and tubular cisternae, and displayed smaller pre-synaptic area. Fewer mitochondria were also observed in post-synaptic dendritic spines in the same study. The loss of these intracellular structures may compromise metabolism and function of synapses in the aged brain. Indeed, the incidence of synapses that contain either no or very few vesicles is largely increased in the aged brain[44]. However, changes in synaptic structures that may lead to improvement of synaptic function have also been reported. For instance, loss of synapses and changes in pre-synaptic structures has been shown to be accompanied by an increase in the mean length of the post-synaptic active zone[50]. Similar increases in the size of pre-synaptic terminals after age-related synaptic loss was observed in the dentate gyrus[51,52].

These structural modifications in the remaining synapses of the aged brain may represent a compensatory phenomenon to maintain normal cortical synaptic function. Age-related decreases in the number of cortical synapses have also been reported[45-47,53,54]. Interestingly, there is also evidence which supports a more profound reduction of both dendrites and synapses in deep cortical layers of aged rats (V, VI) than in superficial layers[55,56]. In our group, the ultrastructural analysis of the rat cerebral cortex revealed a lower density of pre-synaptic terminals per unit length of electrophysiologically characterized and intracellularly post-synaptic membrane of labeled pyramidal neurons in aged brain[24]. Loss of synaptic elements in layer V neocortical pyramidal neurons may result in a decline of function in the aged brain.

Studies of glucose utilization[57] and blood flow[58-60] revealed significant reduction in metabolic activity in the aged cerebral cortex. In addition, profound trimming of dendritic spines in the aged brain may suggest a major loss of asymmetric synapses[29]. Given that these asymmetric synapses have been suggested to be excitatory[28,61,62], age-related synaptic loss may result in a preferential reduction of excitatory rather than inhibitory synaptic inputs to cortical pyramidal neurons.

Although decreases in cortical neuronal activities have been reported[63,64], no major loss in the spontaneous firing rate of layer V pyramidal neurons was shown[63,65]. Interestingly, a similar stability of cellular characteristics in the aged brain has been observed in the hippocampus[66]. One of the possible explanations of this discrepancy is a compensatory functional change in synaptic transmission after loss of synapses. In this regard, our observations indicate that spontaneous axonal firing may compensate for losses in functional synaptic contact on pyramidal neurons in the aged rats. Given the marked shortening of dendrites and decrease in presynaptic bouton density in layer V pyramidal neurons, one would expect a comparable reduction in synaptic input bombarding those neurons. Despite the drastic reduction in synaptic substrate in aged rats, no difference in the frequency of spontaneous action potential-dependant currents (sEPSCs or sIPSCs) between young and aged rats were found (Figure 1). However, in the presence of TTX a clear diminution in the frequency of both mEPSCs and mIPSCs were observed, revealing a TTX insensitive component of synaptic bombardment (Figure 2).

The age-related decrease in the frequency of mIPSCs is consistent with the reduction of the surface area of cell body, which appear to be the primary target of inhibitory GABAergic synapses[29,67]. Because most excitatory synapses appear to occur on dendritic spines[68], the reduction in the spine density is also consistent with the decreased mEPSC frequency we observed, as a preferential loss of asymmetric synapses has been reported[29]. The net result is maintenance of the balance between the two types of inputs.

As no age-related changes has been observed in the frequency of the spontaneous PSCs (either excitatory or inhibitory) despite the structural losses of presynaptic inputs and of diminution of the incidence of miniature PSCs, it can be concluded that the maintenance of these synaptic discharges at normal frequencies reflect a compensatory effort of the aged brain. This can be interpreted as indicating an increase in action potential-dependant input (i.e. an increase in the ratio of sPSC frequency to mPSC frequency) and thus an increased activity in neurons

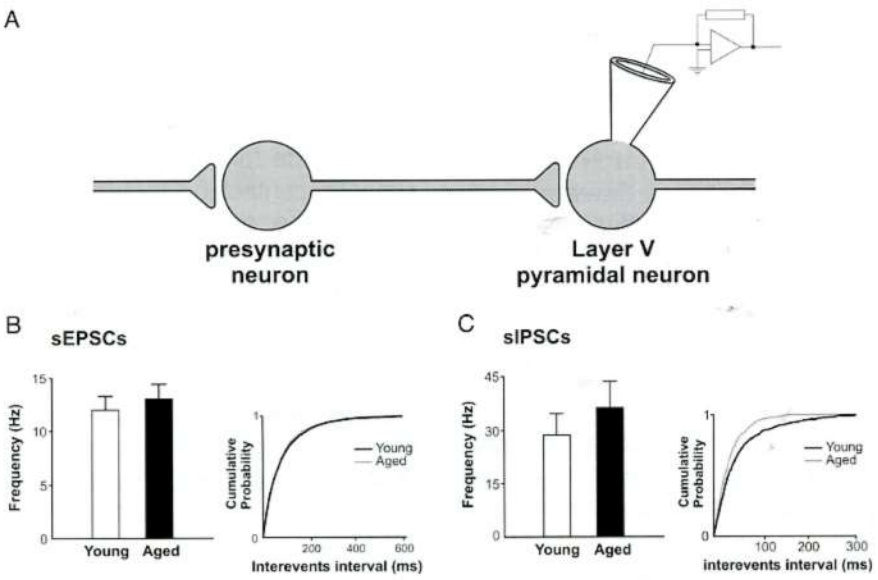


Figure 1. No significant change in the frequency of spontaneously occurring EPSCs and IPSCs in cortical pyramidal neurons of aged rats. *A*. Schematic diagram showing the type of activity reflected by sPSCs recorded in layer V pyramidal neurons (contrast with figure 2*A*.). These sPSCs result from the sum of both the intrinsic releasing properties of the synaptic terminal **and** the action potential firing activity in neurons presynaptic to the recorded cell. *B. C.* Histograms show the frequency of both sEPSCs and sIPSCs from young and aged rats. The cumulative probability plots on the right of each histogram further illustrate the lack of modification in the distribution of frequency of both sEPSCs and sIPSCs with aging. Reproduced, with permission, from Wong et al[24].

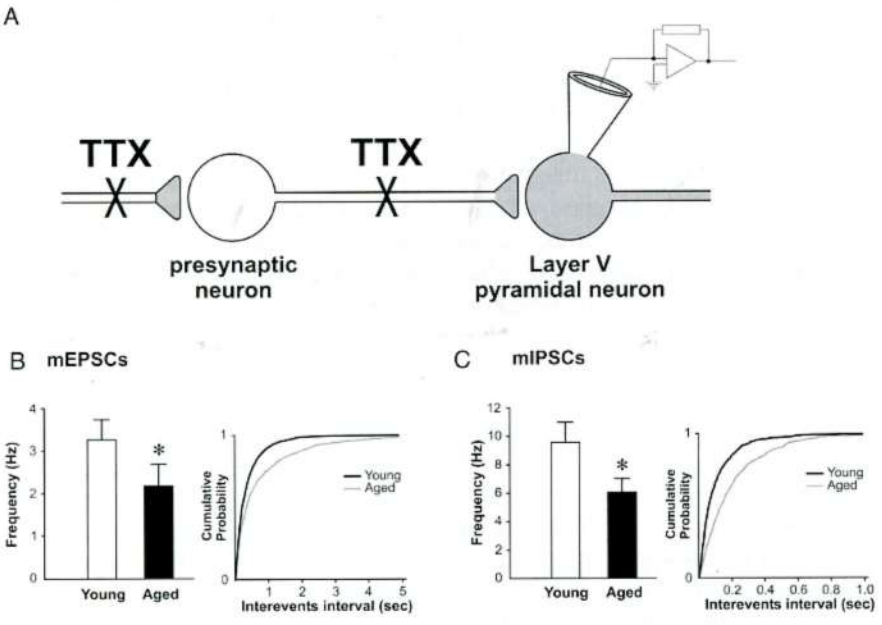


Figure 2. Significant reduction in the frequency of action potential-independent mEPSCs and mIPSCs in cortical pyramidal neurons of aged rats. *A*. Schematic diagram showing the type of activity reflected by mPSCs recorded from layer V pyramidal neurons (contrast with figure 1*A*). With the propagation of action potential being blocked by the addition of 1μM tetrodotoxin (TTX) in the bathing solution, the mPSCs correspond only to action potential independent spontaneous release of neurotransmitter from synaptic terminals effectively uncoupled from the soma-dendritic region of the presynaptic cell. *B*. Histogram displaying the significant 33.3% decrease in the frequency of mEPSCs in aged rats ($p<0.05$) *C*. A similar significant 36.7% decrease in the frequency of mIPSCs in aged rats ($p<0.05$). The graphs on the right are cumulative probability plots of interevent intervals further illustrating the decrease in frequency of mPSCs in aged rats. Reproduced, with permission, from Wong et al[24].

that are presynaptic to pyramidal neurons in aged animals[24].

The basalo-cortical forebrain cholinergic system in aging

Since the number of neurons probably remains rather stable in the aged cerebral cortex, the loss of these pre- and post-synaptic structures would result in a substantial loss of interneuronal connections with aging. The cholinergic innervations of the cerebral cortex has been extensively investigated because of its role in arousal, learning and memory[69-75]. Cholinergic neurons in the nucleus basalis magnocellularis (NBM) and associated forebrain nuclei are the major sources of the extrinsic cholinergic innervations of the cortex[76,77]. In rodents, a small portion of the cortical cholinergic innervation is also derived from intrinsic neurons[78]. The density of cholinergic terminals is particularly high in cortical layer V[79], where pyramidal neurons, the principal output of the cells from this area, are located[28,79]. The role of ACh in memory has become of interest since the learning and memory deficits of aging and AD have been attributed, at least in part, to a decline in the functional integrity of the forebrain cholinergic systems[80-85].

Stronger support for a critical role of cortical ACh in age- and dementia-associated cognitive decline has been provide by studies demonstrating a correlation between decreases in markers of cortical ACh and the severity of dementia[8,86-88]. Several studies support the hypothesis that the integrity of the basal forebrain cholinergic neurons that project to cortical areas is compromised during normal aging and in dementia. With aging, neurons in the basal forebrain undergo a process of atrophy, as judged from morphological and biochemical parameters[2,89].

In addition, preliminary data from our laboratory has demonstrated that there is an age-related loss of cholinergic boutons in the parietal

cortex with a more pronounced decline in layers V and VI[90]. Interestingly, layer V is the area where the highest age-related loss in the total population of synaptic boutons is also observed[55].

More recently, cortical cholinergic terminations have also been regarded as essential to the cerebral cortex's ability to acquire adequate representations in response to sensory experiences[91]. It is ironic that, although ACh was the first neurochemical to be proposed as a CNS transmitter[92-94], it was only in the early eighties that we were able to identify microscopically CNS cholinergic neurons and fibre pathways, thanks to the development of reliable markers for cholinergic neurons, i.e. specific antibodies capable of detecting the ACh biosynthetic enzyme, choline acetyltransferase (ChAT), by immunocytochemistry[25,77,95,96]. A complete understanding of this transmitter system is nowadays part of classical neuropharmacology.

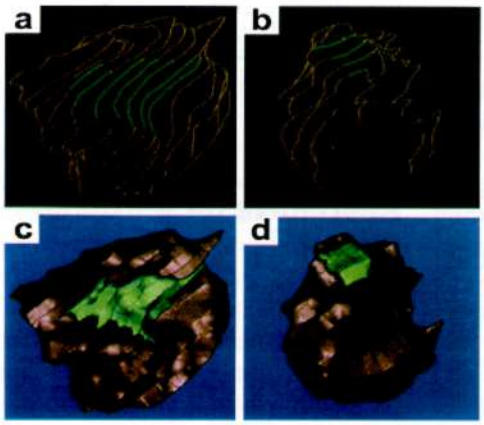
The issue of how ACh acts in the cerebral cortex has been an issue of controversy for some time. Although the electrophysiological data[97,98] clearly points towards a synaptic action of ACh in the CNS, the prevalent opinion has been that this transmitter acted in the cerebral cortex in a diffuse, non-synaptic manner[99-101], a concept falling within the framework of the so-called "volume transmission"[102-104]. These views have been widely supported in recent years.

Recent technological developments and the application of more suitable antibodies to reveal cholinergic neurons have made it possible to derive precise qualitative and quantitative information on cholinergic presynaptic sites. Our studies[105] applying vesicular ACh transporter (VAChT) antibodies and improved immunocytochemical protocols have unequivocally demonstrated that cortical cholinergic boutons (VAChT-immunoreactive) establish, in their majority, classical synapses. These are mostly synapses of the symmetric type terminating on dendritic shafts. These findings strongly support a "classical" (point-to-point) synaptic transmitter role for cortical ACh in opposition to a non-synaptic diffused release modality of action. Furthermore, the same studies indicated a severe age-dependent atrophy (as shown by means of 2D quantification as well as 3D reconstructions) of these cortical presynaptic cholinergic boutons with a concomitant loss of the synaptic area (Figure 3)[105].

The cholinergic neurons located in the NBM (nucleus of Meynert in primates), and associated forebrain nuclei, were shown to be the major sources of the extrinsic cholinergic innervation of the neocortex[76,106]. The density of cholinergic terminals is particularly high in neocortical layer V[79], which is precisely where the larger pyramidal neurons are located[28]. Interestingly, the most recent data from our lab and others supports a more profound reduction in both dendrites[56] and synapses[55] in deep cortical layers (V, VI) of aged rats than in superficial layers. Thus, our recent investigations have shown that the cholinergic presynaptic component is more vulnerable to the aging process than the overall pre-synaptic population[107]. In addition, these correlative studies characterizing layer V pyramidal neurons by intracellular filling with biocytin along with the electron microscopical detection of VAChT demonstrated the preferential association of cholinergic pre-synaptic boutons with pyramidal neurons, in particular with the distal segments of basal dendrites (Figure 4)[107]. In summary, this recent information brings our knowledge of the basalo-cortical cholinergic projection to a new level by providing a detailed account of its presynaptic terminals in the neocortex upon their targets, the pyramidal neurons, where primarily ACh acts.

Trophic support in aged cortical circuits

As discussed in the previous section, the cholinergic system is com-



Morphometric features of VAChT-IR varicosities in layer V of young and aged rats.

	Young	Aged
Isolated sections		
Profile area (μm²)	0.313±0.005	0.241±0.004*
Perimeter (μm)	2.404±0.060	2.052±0.017*
Serial section reconstructions		
Volume (μm³)	0.252±0.046	0.129±0.015*
Surface area (μm²)	2.239±0.326	1.441±0.118*
Synaptic area (μm²)	0.090±0.020	0.040±0.004*

Figure 3. Serial section reconstructions of VAChT-IR boutons in layer V of the parietal cortex of young (a,c) and aged (b,d) rats, obtained with the help of the MCID-M4 image analysis system. Green highlighted areas in the reconstructed boutons represent regions of synaptic contacts. Note the smaller volume and synaptic area in the aged boutons compared to young. Scale bar, 0.5 μm. The values shown in the table represent the mean of each parameter per animal ± S.E.M. *p <0.05; Student's t-test. Reproduced, with permission, from Turrini et al[105].

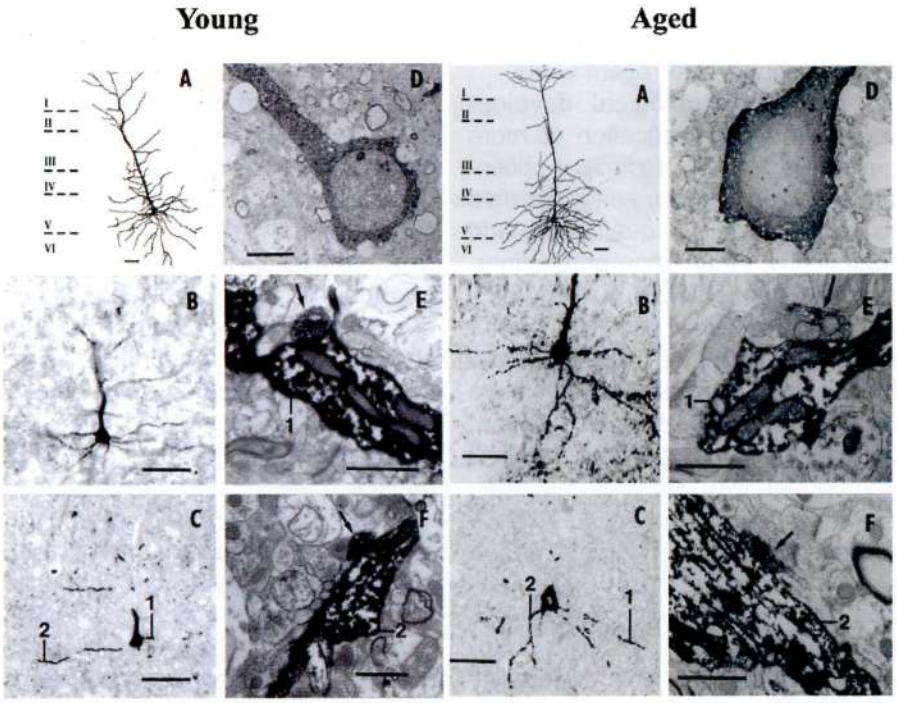


Figure 4. Morphological and immunocytochemical properties of a biocytin-labeled lamina V pyramidal neuron from a young (on the left, A to F) and aged rat (on the right, A to F). (A) Camera lucida reconstruction of the cell. (B) Micrograph of part of the cell obtained from a 50µm thick plastic section. (C) Micrograph from a 4µm thick plastic section obtained after Epon re-embedding of the 50µm thick section from B. (D) Electron micrograph of the cell body area obtained after further re-embedding the 4µm thick shown in (C). Note the absence of VAChT-IR boutons apposed to the cell body and elsewhere in the surrounding neuropile (E,F). Electron micrographs of proximal and distal dendrites, respectively; note the VAChT-IR boutons apposed to the dendrites (arrows). Scale bars in (A), (B) and (C) = 5µm; (E) and (F) = 1µm. Reproduced, with permission, from Casu et al[107].

promised in aging and, even more so, in AD[81,83,108]. The selective cholinergic involvement in AD has been recently replicated by us in transgenic animals reproducing features of the amyloid pathology[24]. It would follow from our knowledge of the exquisite NGF dependence of the forebrain cholinergic phenotype that both in aging and in AD pathology a failure of the trophic factor feedback is responsible for the cholinergic decline occurring in such circumstances. However, the evidence for a decreased production of NGF in aging or AD is not compelling.

Communications regarding age-dependent changes in NGF protein levels or its mRNA in the cerebral cortex and hippocampus vary from a decrease, to no change or to an elevation and, in many occasions, a dissociation of these two markers[109-113]. Early results obtained by Gage, Bkjorklund and Thoenen[114] reported that behaviorally impaired rats display normal or elevated NGF levels in the CNS, which, based on the available information, we would interpret as a possible increase in Pro-NGF (immature NGF) material rather than NGF itself.

The NGF status in AD is also puzzling as there is an apparent dissociation between the classical markers of this neurotrophin (the peptide and its mRNA content) and the phenotypic state of forebrain cholinergic neurons. Thus, as elegantly demonstrated by Fahnestock and collaborators, in post-mortem cortical samples of AD the NGF peptide content is elevated[115,116] while there is no change in the corresponding mRNA[115]. This situation would imply a failure in NGF processing and maturation. The observation of elevated NGF peptides and normal levels of NGF mRNA in the AD brain has been confirmed by others[117,118]. In addition, Fahnestock and collaborators[119] have shown that immunoassayable NGF in the mouse, rat and human brains is largely represented by the precursor, immature form of the NGF protein. This elevation of Pro-NGF levels might explain the trophic uncoupling of forebrain cholinergic neurons in AD.

In AD, along with the well-known loss of nucleus basalis neurons[84] or their atrophy[120], several authors have found a decrease in the retrogradely transported NGF protein in this forebrain region (by ELISA) or in the nucleus basalis cells (by immunocytochemistry)[121,122]. This situation has been attributed to a failure of the NGF retrograde axonal transport system from the cerebral cortex to the cell soma[123-125]. As the TrkA receptor appears down regulated in the nucleus basalis of AD patients and aged rats[118,124,126,127], the trophic disconnection could be due to the inability of cholinergic terminals to bind and internalize TrkA/NGF complexes. However, it is also possible that these conditions (i.e. a lack of NGF transport and the down regulation of TrkA receptors) are related to a single factor: the failure of the maturation of Pro-NGF into a biologically active trophic molecule. One factor favouring such an interpretation is that the lesion-induced trophic disconnection of the nucleus basalis[128] or the septal nucleus[129] leads to a down regulation of the TrkA mRNA message. The down regulated trkA message in atrophic neurons in both models (septum and nucleus basalis) still respond positively to the *exogenous* administration of NGF by up-regulating the TrkA mRNA message[129,130].

Alternative for a trophic therapy?

Our observations have shown that either provoking the immunoneutralization of *endogenous* NGF or the blockade of their TrkA receptors, results in the loss of pre-existing cortical cholinergic pre-synaptic sites. This is a strong argument favouring a role of *endogenous* NGF in the maintenance of the steady-state

number of cholinergic connections in mature animals[131].

We have also shown that synthetic cyclic-peptides mimicking the 92-96 loop (β turn) of the NGF molecule act as antagonists to the TrkA receptor molecule, modulating cholinergic phenotype *in vitro*[132] as well as *in vivo*[131]. As a result, we investigated the ability of a new small molecular weight agent, named D3[133], to alter the atrophic cortical cholinergic pre-synaptic terminations in age-impaired rats in the search for alternative therapeutic compounds affecting *solely* the TrkA neurotrophin receptor without cross-binding to the ubiquitous $p75^{LNTR}$ low affinity receptor. As a result of an intracerebroventricular delivery of D3 to cognitively impaired rats, we found a marked increase in the number of the pre-synaptic cholinergic boutons in the cerebral cortex of aged rats. Moreover, D3 treatment for two weeks was capable of reverse the spatial learning impairment displayed by a small portion of 24 months old Fischer-344 rats, using the Morris water maze task (Bruno et al., submitted).

Conflicting results may also arise from the fact that the aged populations represent a heterogeneous group with varying levels of cognitive deficits. Our preliminary results indicate that separation of aged animals into memory impaired vs. unimpaired reveals potentially dramatic differences in synaptic elements between the two groups. Furthermore, our results not only point to a differential atrophy of synapses between the two groups of aged animals but also, and perhaps more importantly, to a differential atrophy between neurotransmitter systems (cholinergic *vs.* non-cholinergic and excitatory *vs.* inhibitory), and thus a change in the balance of inputs to specific cell populations may be a prominent factor affecting cognitive performance in aged individuals.

The question which remains is whether this age-related cholinergic atrophy is a widespread predicament in aging, or is it more pronounced or selective to aged impaired animals. We hypothesize that severe structural cortical cholinergic synaptic deterioration should tilt the balance of CNS transmitter functions negatively leading to age-related cognitive impairments.

Concluding remarks

Our investigations revealed that a major shrinkage of large pyramidal neurons takes place with aging. Furthermore, that there is a close correlation between these loses of presynaptic input to morphologically and electrophysiological characterized pyramidal neurons with TTX insensitive synaptic current (excitatory and inhibitory). We have further demonstrated that a compensatory synaptic activity occurs in aged cerebral cortex. Our investigations also revealed that most of the synaptic losses occur at deep cortical layers and preferentially affect cholinergic inputs.

Cholinergic presynaptic boutons terminate mostly in pyramidal neurons and develop an age-related atrophy along with losses of synaptic contacts. These cholinergic synaptic contacts are dependant upon *endogenous* NGF secretions and they can be rescue in aged cognitively impaired rats with the application of *exogenous* NGF or NGF-derivatives.

Acknowledgements

This review was supported by grands from the CIHR (MOP-62735) to ACC and the NIH (R01AG20529) to ACC, ARS, and YDK. The authors would like to acknowledge the contributions made to the original research behind this review by Maria Antonietta Casu, Paolo Turrini, Uri Saragovi and Tak Pan Wong. The authors would like to thank Jean Emmanuel Tsang and Alan Forster for illustration expertise and Sid Parkinson for his assistance with editorial matters.

References

1. Williams L.R., Rylett R.J., Ingram D.K., Joseph J.A., Moises H.C., Tang A.H., and Mervis R.F., NGF affects the cholinergic biochemistry and behaviour of aged rats. *Prog. Brain Res.* 1993; 98: 241-250.
2. Rylett R.J. and Williams L.R., Role of neurotrophins in cholinergic-neurone function in the adult and aged CNS. *Trends Neurosci.* 1994; 17: 486-490.
3. Michalek H., Fortuna S., and Pintor A., Age-related differences in brain choline acetyltransferase, cholinesterase and muscarinic recptor sites in two strains of rats. *Neurobiol. Aging* 1989; 10: 143-148.
4. Meyer E.M., St Onge E., and Crews F.T., Effects of aging on rat cortical presynaptic cholinergic processes. *Neurobiol. Aging* 1984; 5: 315-317.
5. Armstrong D.M., Hersh L.B., and Gage F.H., Morphologic alterations of cholinergic processes in the neocortex of aged rats. *Neurobiol. Aging* 1988; 9: 199-205.
6. Fischer W., Chen K.S., Gage F.H., and Bjorklund A., Progressive decline in spatial learning and integrity of forebrain cholinergic neurons in rats during aging. *Neurobiol. Aging* 1992; 13: 9-23.
7. Chen K.S., Masliah E., Mallory M., and Gage F.H., Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human nerve growth factor infusion. *Neuroscience* 1995; 68: 19-27.
8. Bigl V., Arendt T., Fischer S., Fischer S., Werner M., and Arendt A., The cholinergic system in aging. *Gerontology* 1987; 33: 172-180.
9. Smith D.E., Roberts J., Gage F.H., and Tuszynski M.H., Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy. *Proc. Natl. Acad. Sci.* U. S. A 1999; 96: 10893-10898.
10. Brody H., Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. *J. Comp Neurol.* 1955; 102: 511-516.
11. Dekaban A.S., Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. *Ann. Neurol.* 1978; 4: 345-356.
12. Matsumae M., Kikinis R., Morocz I.A., Lorenzo A.V., Sandor T., Albert M.S., Black P.M., and Jolesz F.A., Age-related changes in intracranial compartment volumes in normal adults assessed by magnetic resonance imaging. *J. Neurosurg.* 1996; 84: 982-991.
13. Raz N., Briggs S.D., Marks W., and Acker J.D., Age-related deficits in generation and manipulation of mental images: II. The role of dorsolateral prefrontal cortex. *Psychol. Aging* 1999; 14: 436-444.
14. Raz N., Gunning-Dixon F.M., Head D., Dupuis J.H., and Acker J.D., Neuroanatomical correlates of cognitive aging: evidence from structural magnetic resonance imaging. *Neuropsychology.* 1998; 12: 95-114.
15. Henderson G., Tomlinson B.E., and Gibson P.H., Cell counts in human cerebral cortex in

normal adults throughout life using an image analysing computer. J. Neurol. Sci. 1980; 46: 113-136.
16. Terry R.D., DeTeresa R., and Hansen L.A., Neocortical cell counts in normal human adult aging. *Ann. Neurol.* 1987; 21: 530-539.
17. Brody H., Aging of the vertebrate brain. In: Rockstein M. and Sussman H.M., eds, *Development and aging in the nervous system*. New York: Academic Press, 1973: pp. 121-133.
18. DeKosky S.T. and Bass N.H., Effects of aging and senile dementia on the microchemical pathology of human cerebral cortex. In: Amaducci L., Davison A.N., and Antuono P., eds, *Aging*. New York: Raven Press, 1980: pp. 33-37.
19. Haug H., Knebel G., Mecke E., Orun C., and Sass N.L., The aging of cortical cytoarchitectonics in the light of stereological investigations. *Prog. Clin. Biol.* Res. 1981; 59B: 193-197.
20. Haug H. and Eggers R., Morphometry of the human cortex cerebri and corpus striatum during aging. *Neurobiol. Aging* 1991; 12: 336-338.
21. Peters A., The absence of significant neuronal loss from cerebral cortex with age. *Neurobiol. Aging* 1993; 14: 657-658.
22. Gomez-Isla T., Price J.L., McKeel D.W., Jr., Morris J.C., Growdon J.H., and Hyman B.T., Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci.* 1996; 16: 4491-4500.
23. Brizzee K.R., Sherwood N., and Timiras P.S., A comparison of cell populations at various depth levels in cerebral cortex of young adult and aged Long-Evans rats. *J. Gerontol.* 1968; 23: 289-297.
24. Wong T.P., Marchese G., Casu M.A., Ribeiro-da-Silva A., Cuello A.C., and De Koninck Y., Loss of presynaptic and postsynaptic structures is accompanied by compensatory increase in action potential-dependent synaptic input to layer V neocortical pyramidal neurons in aged rats. *J. Neurosci.* 2000; 20: 8596-8606.
25. Cuello A.C. and Sofroniew M.V., The anatomy of CNS cholinergic neurons. *Trends Neurosci.* 1984; 7: 74-78.
26. Mungai J.M., Dendritic patterns in the somatic sensory cortex of the cat. *J. Anat.* 1967; 101: 403-418.
27. Sholl D.A., The surface area of cortical neurons. *J. Anat.* 1955; 89: 571-572.
28. DeFelipe J. and Farinas I., The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog. Neurobiol.* 1992; 39: 563-607.
29. White E.L., *Cortical circuits: synaptic organization of the cerebral cortex — structure, function and theory.* Boston: Birkhauser, 1989.
30. Jones E.G., Laminar distribution of cortical efferent cells. In: Peters A. and Jones E.G., eds, *Cerebral cortex*. New York: Plenum Press, 1984: pp. 521-533.
31. Scheibel M.E., Lindsay R.D., Tomiyasu U., and Scheibel A.B., Progressive dendritic changes in aging human cortex. *Exp. Neurol.* 1975; 47: 392-403.
32. Scheibel M.E., Tomiyasu U., and Scheibel A.B., The aging human Betz cell. *Exp. Neurol.* 1977; 56: 598-609.
33. Anderson B. and Rutledge V., Age and hemisphere effects on dendritic structure. *Brain* 1996; 119 (Pt 6): 1983-1990.
34. Mervis R., Structural alterations in neurons of aged canine neocortex: a Golgi study. *Exp. Neurol.* 1978; 62: 417-432.
35. Cupp C.J. and Uemura E., Age-related changes in prefrontal cortex of Macaca mulatta: quantitative analysis of dendritic branching patterns. *Exp. Neurol.* 1980; 69: 143-163.
36. Jacobs B. and Scheibel A.B., A quantitative dendritic analysis of Wernicke's area in humans. I. Lifespan changes. *J. Comp Neurol.* 1993; 327: 83-96.
37. Leuba G., Aging of dendrites in the cerebral cortex of the mouse. *Neuropathol. Appl. Neurobiol.* 1983; 9: 467-475.
38. Vaughan D.W., Age-related deterioration of pyramidal cell basal dendrites in rat auditory cortex. *J. Comp Neurol.* 1977; 171: 501-515.
39. Feldman M.L., Dendritic changes in aging rat brain: pyramidal cell dendrite length and ultrastructure. In: Nandy K. and Sherwin I., eds, *The Aging Brain and Senile Dementia*. New York: Plenum Press, 1977: pp. 23-37.
40. Coleman P.D. and Flood D.G., Net dendritic stability of layer II pyramidal neurons in F344 rat entorhinal cortex from 12 to 37 months. *Neurobiol. Aging* 1991; 12: 535-541.
41. 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: 661-680.
42. Mervis R.F., Pope D., Lewis R., Dvorak R.M., and Williams L.R., Exogenous nerve growth factor reverses age-related structural changes in neocortical neurons in the aging rat. A quantitative Golgi study. *Ann. NY Acad.* Sci. 1991; 640: 95-101.
43. Sholl D.A., The organization of the visual cortex in the cat. *J. Anat.* 1955; 89: 33-46.
44. Adams I. and Jones D.G., Quantitative ultrastructural changes in rat cortical synapses during early-, mid- and late-adulthood. *Brain Res.* 1982; 239: 349-363.
45. Zecevic N., Bourgeois J.P., and Rakic P., Changes in synaptic density in motor cortex of rhesus monkey during fetal and postnatal life. Brain Res. Dev. *Brain Res.* 1989; 50: 11-32.
46. Adams I., Comparison of synaptic changes in the precentral and postcentral cerebral cortex of aging humans: a quantitative ultrastructural study. *Neurobiol. Aging* 1987; 8: 203-212.
47. Huttenlocher P.R., Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res.* 1979; 163: 195-205.
48. Geinisman Y., Toledo-Morrell L., Morrell F., Persina I.S., and Rossi M., Age-related loss of axospinous synapses formed by two afferent systems in the rat dentate gyrus as revealed by the unbiased stereological dissector technique. *Hippocampus* 1992; 2: 437-444.
49. Lolova I., Lolov V., Itsev D., and Petkov V.D., Changes in the synapses in the parietal cortex of senescent rats. *Acta Physiol Pharmacol. Bulg.* 1990; 16: 42-49.
50. Adams I., Plasticity of the synaptic contact zone following loss of synapses in the cerebral cortex of aging humans. *Brain Res.* 1987; 424: 343-351.
51. Bertoni-Freddari C., Giuli C., Pieri C., and Paci D., Age-related morphological rearrangements of synaptic junctions in the rat cerebellum and hippocampus. *Arch. Gerontol. Geriatr.* 1986; 5: 297-304.
52. Bertoni-Freddari C., Fattoretti P., Paoloni R., Caselli U., Galeazzi L., and Meier-Ruge W., Synaptic structural dynamics and aging. *Gerontology* 1996; 42: 170-180.
53. Markus E.J. and Petit T.L., Neocortical synaptogenesis, aging, and behavior: lifespan development in the motor-sensory system of the rat. *Exp. Neurol.* 1987; 96: 262-278.
54. Bourgeois J.P. and Rakic P., Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *J. Neurosci.* 1993; 13: 2801-2820.
55. Wong T.P., Campbell P.M., Ribeiro-da-Silva A., and Cuello A.C., Synaptic numbers across cortical laminae and cognitive performance of the rat during ageing. *Neuroscience* 1998; 84: 403-412.
56. de Brabander J.M., Kramers R.J., and Uylings H.B., Layer-specific dendritic regression of pyramidal cells with ageing in the human prefrontal cortex. *Eur. J. Neurosci.* 1998; 10: 1261-1269.
57. Leenders K.L., Perani D., Lammertsma A.A., Heather J.D., Buckingham P., Healy M.J., Gibbs J.M., Wise R.J., Hatazawa J., Herold S., and ., Cerebral blood flow, blood volume and oxygen utilization. Normal values and effect of age. *Brain* 1990; 113 (Pt 1): 27-47.
58. Gur R.C., Gur R.E., Obrist W.D., Skolnick B.E., and Reivich M., Age and regional cerebral blood flow at rest and during cognitive activity. *Arch. Gen. Psychiatry* 1987; 44: 617-621.
59. Melamed E., Lavy S., Bentin S., Cooper G., and Rinot Y., Reduction in regional cerebral blood flow during normal aging in man. *Stroke* 1980; 11: 31-35.
60. Shaw T.G., Mortel K.F., Meyer J.S., Rogers R.L., Hardenberg J., and Cutaia M.M., Cerebral blood flow changes in benign aging and cerebrovascular disease. *Neurology* 1984; 34: 855-862.
61. Uchizono K., Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. *Nature* 1965; 207: 642-643.
62. Gray E.G., Electron microscopy of excitatory and inhibitory synapses: a brief review. *Prog. Brain Res.* 1969; 31: 141-155.
63. Stern W.C., Pugh W.W., and Morgane P.J., Single unit activity in frontal cortex and caudate nucleus of young and old rats. *Neurobiol. Aging* 1985; 6: 245-248.
64. Roy D. and Singh R., Age-related change in the multiple unit activity of the rat brain parietal cortex and the effect of centrophenoxine. *Exp. Gerontol.* 1988; 23: 161-174.

65. Lamour Y., Dutar P., and Jobert A., Cerebral neocortical neurons in the aged rat: spontaneous activity, properties of pyramidal tract neurons and effect of acetylcholine and cholinergic drugs. *Neuroscience* 1985; 16: 835-844.
66. Barnes C.A., Normal aging: regionally specific changes in hippocampal synaptic transmission. *Trends Neurosci.* 1994; 17: 13-18.
67. Soltesz I., Smetters D.K., and Mody I., Tonic inhibition originates from synapses close to the soma. *Neuron* 1995; 14: 1273-1283.
68. Feldman M.L., Morphology of the neocortical pyramidal neuron. In: Peters A. and Jones E.G., eds, *Cerebral Cortex*, Vol. 1. Cellular Components of the Cerebral Cortex. New York: Plenum Press, 1984: pp. 123-200.
69. Bartus R.T., Dean R.L., Pontecorvo M.J., and Flicker C., The cholinergic hypothesis: a historical overview, current perspective, and future directions. *Ann. N. Y. Acad. Sci.* 1985; 444: 332-358.
70. Hasselmo M.E., Neuromodulation: acetylcholine and memory consolidation. *Trends Cogn Sci.* 1999; 3: 351-359.
71. McCormick D.A., Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends Neurosci.* 1989; 12: 215-221.
72. McCormick D.A., Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog. Neurobiol.* 1992; 39: 337-388.
73. Metherate R., Cox C.L., and Ashe J.H., Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J. Neurosci.* 1992; 12: 4701-4711.
74. Olton D., Markowska A., Voytko M.L., Givens B., Gorman L., and Wenk G., Basal forebrain cholinergic system: A functional analysis. *Adv. Exp. Med. Biol.* 1991; 295: 353-372.
75. Voytko M.L., Olton D.S., Richardson R.T., Gorman L.K., Tobin J.R., and Price D.L., Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *J. Neurosci.* 1994; 14: 167-186.
76. Fibiger H.C., The organization and some projections of cholinergic neurons of the mammalian forebrain. *Brain Res. Rev.* 1982; 4: 327-388.
77. Mesulam M.-M., Mufson E.J., Levey A.I., and Wainer B.H., Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. Comp. Neurol.* 1983; 214: 170-197.
78. Eckenstein F. and Baughman R.W., Two types of cholinergic innervation in cortex, one co-localized with vasoactive intestinal polypeptide. *Nature* 1983; 309: 153-155.
79. Houser C.R., Crawford G.D., Salvaterra P.M., and Vaughn J.E., Immunocytochemical localization of choline acetyltransferase in rat cerebral cortex: A study of cholinergic neurons and synapses. *J. Comp. Neurol.* 1985; 234: 17-34.
80. Davies P. and Maloney A.J.F., Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* 1976; 2 (8000): 1403.
81. Bowen D.M., Smith C.B., White P., and Davison A.N., Neurotransmitter related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain* 1976; 99: 459-496.
82. Bartus R.T., Dean R.L., Beer B., and Lippa A.S., The cholinergic hypothesis of geriatric memory dysfunction. *Science* 1982; 217: 408-417.
83. Whitehouse P.J., Price D.L., Clark A.W., Coyle J.T., and DeLong M.R., Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* 1981; 10: 122-126.
84. Whitehouse P.J., Price D.L., Struble R.G., Clark A.W., Coyle J.T., and DeLong M.R., Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* 1982; 215: 1237-1239.
85. Coyle J.T., Price D.L., and DeLong M.R., Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* 1983; 219: 1184-1190.
86. McGeer P.L., McGeer E.G., Suzuki J., Dolman C.E., and Nagai T., Aging, Alzheimer's disease, and the cholinergic system of the basal forebrain. *Neurology* 1984; 34: 741-745.
87. Sparks D.L., Hunsaker J.C., III, Slevin J.T., DeKosky S.T., Kryscio R.J., and Markesbery W.R., Monoaminergic and cholinergic synaptic markers in the nucleus basalis of Meynert (nbM): Normal age-related changes and the effect of heart disease and Alzheimer's disease. *Ann. Neurol.* 1992; 31: 611-620.
88. Etienne P., Robitaille Y., Wood P., Gauthier S., Nair N.P., and Quirion R., Nucleus basalis neuronal loss, neuritic plaques and choline acetyltransferase activity in advanced Alzheimer's disease. *Neuroscience* 1986; 19: 1279-1291.
89. Fischer W., Björklund A., Chen K., and Gage F.H., NGF improves spatial memory in aged rodents as a function of age. *J. Neurosci.* 1991; 11: 1889-1906.
90. Marchese, G., Wong, T. P., Ribeiro-da-Silva, A., and Cuello, A. C., *Age-related depletion of cholinergic synapses in the rat cerebral cortex.* Society for Neuroscience abstracts. 1998; 24: 1493.
91. Kilgard M.P. and Merzenich M.M., Cortical map reorganization enabled by nucleus basalis activity. *Science* 1998; 279: 1714-1718.
92. Feldberg W., The chemical transmitter at synapses in a sympathetic ganglion. *J. Physiol* 1976; 263: 89P-91P.
93. Feldberg W., Recent views on the mode of action of acetylcholine in the central nervous system. *Physiol. Rev.* 1945; 25: 596-542.
94. Vogt M., Physiological aspects of cholinergic and monoaminergic neurones. *J. Physiol* 1969; 201: 3P.
95. Eckenstein F., Barde Y.A., and Thoenen H., Production of specific antibodies to choline acetyltransferase purified from pig brain. *Neuroscience* 1981; 6: 993-1000.
96. Sofroniew M.V., Eckenstein F., Thoenen H., and Cuello A.C., Topography of choline acetyltransferase-containing neurons in the forebrain of the rat. *Neurosci. Lett.* 1982; 33: 7-12.
97. McCormick D.A., Cellular mechanisms underlying cholinergic and noradrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. *J. Neurosci.* 1992; 12: 278-289.
98. Krnjevic K., Central cholinergic mechanisms and function. *Prog. Brain Res.* 1993; 98: 285-292.
99. Descarries L., The hypothesis of an ambient level of acetylcholine in the central nervous system. *J. Physiol Paris* 1998; 92: 215-220.
100. Descarries L., Gisiger V., and Steriade M., Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* 1997; 53: 603-625.
101. Mechawar N., Cozzari C., and Descarries L., Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. *J. Comp Neurol.* 2000; 428: 305-318.
102. Jansson A., Tinner B., Bancila M., Verge D., Steinbusch H.W., Agnati L.F., and Fuxe K., Relationships of 5-hydroxytryptamine immunoreactive terminal-like varicosities to 5-hydroxytryptamine-2A receptor-immunoreactive neuronal processes in the rat forebrain. *J. Chem. Neuroanat.* 2001; 22: 185-203.
103. Zoli M., Torri C., Ferrari R., Jansson A., Zini I., Fuxe K., and Agnati L.F., The emergence of the volume transmission concept. *Brain Res. Rev.* 1998; 26: 136-147.
104. Agnati L.F., Zoli M., Stromberg I., and Fuxe K., Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience* 1995; 69: 711-726.
105. Turrini P., Casu M.A., Wong T.P., De Koninck Y., Ribeiro-da-Silva A., and Cuello A.C., Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. *Neuroscience* 2001; 105: 277-285.
106. Mesulam M.-M., Mufson E.J., Wainer B.H., and Levey A.I., Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience* 1983; 10: 1185-1201.
107. Casu M.A., Wong T.P., De Koninck Y., Ribeiro-da-Silva A., and Cuello A.C., Aging causes a preferential loss of cholinergic innervation of characterized neocortical pyramidal neurons. *Cereb. Cortex* 2002; 12: 329-337.
108. Davies P. and Maloney A., A selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* 1976; 2: 1403.
109. Alberch J., Pérez-Navarro E., Arenas E., and Marsal J., Involvement of nerve growth factor and its receptor in the regulation of the cholinergic function in aged rats. *J. Neurochem.* 1991; 57: 1483-1487.
110. Henriksson B.G., Soderstrom S., Gower A.J., Ebendal T., Winblad B., and Mohammed A.H., Hippocampal nerve growth factor levels are related to spatial learning ability in aged rats. *Behav. Brain Res.* 1992; 48: 15-20.
111. Katoh-Semba R., Semba R., Takeuchi I.K., and Kato K., Age-related changes in levels of brain-derived neurotrophic factor in selected brain regions of rats, normal mice and senescence-accelerated mice: a comparison to those of nerve growth factor and

neurotrophin-3. *Neurosci. Res.* 1998; 31: 227-234.

112. Larkfors L., Ebendal T., Whittemore S.R., Persson H., Hoffer B., and Olson L., Decreased level of nerve growth factor (NGF) and its messenger RNA in the aged rat brain. *Brain Res.* 1987; 427: 55-60.
113. Larkfors L., Ebendal T., Whittemore S.R., Persson H., Hoffer B., and Olson L., Developmental appearance of nerve growth factor in the rat brain: significant deficits in the aged forebrain. *Prog. Brain Res.* 1988; 78: 27-31.
114. Hellweg R., Fischer W., Hock C., Gage F.H., Björklund A., and Thoenen H., Nerve growth factor levels and choline acetyltransferase activity in the brain of aged rats with spatial memory impairments. *Brain Res.* 1990; 537: 123-130.
115. Jette N., Cole M.S., and Fahnestock M., NGF mRNA is not decreased in frontal cortex from Alzheimer's disease patients. Brain Res. Mol. *Brain Res.* 1994; 25: 242-250.
116. Fahnestock M., Scott S.A., Jette N., Weingartner J.A., and Crutcher K.A., Nerve growth factor mRNA and protein levels measured in the same tissue from normal and Alzheimer's disease parietal cortex. *Brain Res Mol Brain Res* 1996; 42: 175-178.
117. Hock C., Heese K., Hulette C., Rosenberg C., and Otten U., Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch. Neurol.* 2000; 57: 846-851.
118. Hock C., Heese K., Muller-Spahn F., Hulette C., Rosenberg C., and Otten U., Decreased trkA neurotrophin receptor expression in the parietal cortex of patients with Alzheimer's disease. *Neurosci.* Lett. 1998; 241: 151-154.
119. Fahnestock M., Michalski B., Xu B., and Coughlin M.D., The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Mol. Cell Neurosci.* 2001; 18: 210-220.
120. Pearson R.C., Sofroniew M.V., Cuello A.C., Powell T.P., Eckenstein F., Esiri M.M., and Wilcock G.K., Persistence of cholinergic neurons in the basal nucleus in a brain with senile dementia of the Alzheimer's type demonstrated by immunohistochemical staining for choline acetyltransferase. *Brain Res.* 1983; 289: 375-379.
121. Scott S.A., Mufson E.J., Weingartner J.A., Skau K.A., and Crutcher K.A., Nerve growth factor in alzheimers disease - increased levels throughout the brain coupled with declines in nucleus basalis. *J. Neurosci.* 1995; 15: 6213-6221.
122. Siegel G.J. and Chauhan N.B., Neurotrophic factors in Alzheimer's and Parkinson's disease brain. *Brain Res. Brain Res. Rev.* 2000; 33: 199-227.
123. De Lacalle S., Cooper J.D., Svendsen C.N., Dunnett S.B., and Sofroniew M.V., Reduced retrograde labelling with fluorescent tracer accompanies neuronal atrophy of basal forebrain cholinergic neurons in aged rats. *Neuroscience* 1996; 75: 19-27.
124. Mufson E.J., Conner J.M., and Kordower J.H., Nerve growth factor in Alzheimer's disease: Defective retrograde transport to nucleus basalis. *NeuroReport* 1995; 6: 1063-1066.
125. Mufson E.J., Kroin J.S., Sendera T.J., and Sobreviela T., Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. *Prog. Neurobiol.* 1999; 57: 451-484.
126. Cooper J.D., Lindholm D., and Sofroniew M.V., Reduced transport of [125I] nerve growth factor by cholinergic neurons and down-regulated TrkA expression in the medial septum of aged rats. *Neuroscience* 1994; 62: 625-629.
127. Mufson E.J., Lavine N., Jaffar S., Kordower J.H., Quirion R., and Saragovi H.U., Reduction in p140-TrkA receptor protein within the nucleus basalis and cortex in Alzheimer's disease. *Exp. Neurol.* 1997; 146: 91-103.
128. Figueiredo B.C., Pluss K., Skup M., Otten U., and Cuello A.C., Acidic FGF induces and its mRNA in the injured neocortex of adult animals. *Mol Brain Res* 1995; 33: 1-6.
129. Venero J.L., Knüsel B., Beck K.D., and Hefti F., Expression of neurotrophin and trk receptor genes in adult rats with fimbria transections: Effect of intraventricular nerve growth factor and brain-derived neurotrophic factor administration. *Neuroscience* 1994; 59: 797-815.
130. Figueiredo B.C., Skup M., Bedard A.M., Tetzlaff W., and Cuello A.C., Differential expression of p140trk, p75NGFR and growth- associated phosphoprotein-43 genes in nucleus basalis magnocellularis, thalamus and adjacent cortex following neocortical infarction and nerve growth factor treatment. *Neuroscience* 1995; 68: 29-45.
131. Debeir T., Saragovi H.U., and Cuello A.C., A nerve growth factor mimetic TrkA antagonist causes withdrawal of cortical cholinergic boutons in the adult rat. *Proc. Natl. Acad. Sci. U. S. A* 1999; 96: 4067-4072.
132. Debeir T., Saragovi H.U., and Cuello A.C., TrkA antagonists decrease NGF-induced ChAT activity in vitro and modulate cholinergic synaptic number in vivo. *J. Physiol.* (Paris) 1998; 92: 205-208.
133. Maliartchouk S., Feng Y., Ivanisevic L., Debeir T., Cuello A.C., Burgess K., and Saragovi H.U., A designed peptidomimetic agonistic ligand of TrkA nerve growth factor receptors. *Mol. Pharmacol.* 2000; 57: 385-391.

Alzheimer's Disease: a TrueTauopathy Fueled by APP Disfunction

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Abstract

At present time, the initiating cause of neurodegeneration in Alzheimer's Disease (AD) is still a matter of debate. A number of hypotheses are proposed including the extracellular neurotoxicity of Aβ or oligomers, the intracellular one, or a loss of function of APP. However, the amyloid cascade hypothesis reflects the major trend of research of the last decade, leaving tau pathology as a possible late consequence. Our biochemical spatiotemporal analysis of tau pathology in non-demented elderly patients from a prospective and multidisciplinary approach corroborates neuropathological findings. Indeed, tau pathology spreads progressively, invariably, hierarchically, from the transentorhinal cortex to the whole neocortex, along cortico-cortical connections. We have shown that tau pathology develops in parallel to Aβ deposition, and is even sometimes present is absence of Aβ deposits. The fact that tau pathology is a neuron-to-neuron spreading phenomenon, and that the neocortical involvement is always found in the presence of Aβ deposits demonstrate that: (1) AD is a real tauopathy; (2) There is a synergy between amyloidosis and tauopathy; (3) The early transformation and decrease of APP carboxy-terminal fragments (APP-CTFs), in parallel to tau pathology, is in favor of a loss of APP function as the central cause of AD. It could be explained by the loss of APP-CTFs trophic activities, provoking the extent of tau pathology. The alternative hypothesis is a neurotoxic effect of N-truncated Aβ species, that aggregate before full lenght Aβ.

1. Alzheimer's Disease: the conjunction of two degenerative processes

Since the beginning of last century, it is well known that AD is characterized by the presence of two neuropathological hallmarks that are amyloid deposits and neurofibrillary tangles[1]. These lesions are distributed all over the human cortex and constitute the basement for neuropathological diagnosis[2]. In the mid eighties the molecular components of both lesions have been discovered.

The extracellular amyloid deposits where demonstrated to be composed of the aggregation of a small peptide named amyloid-beta peptide (βA4 or Aβ)[3]. The Aβ peptide derives from the complex catabolism of a larger precursor named Amyloid Precursor Protein (APP)[4].

The intraneuronal pathological fibrillar structures that constitute neurofibrillary tangles were shown to be made of the aggregation of abnormally modified microtubule-associated tau proteins[5-10].

Indeed, these molecules are not related and thus AD can be considered as the conjunction two independent neurodegenerative processes that are tau and APP pathologies, for which the pathophysiological relationship remains to be established.

2. The aetiology of Alzheimer's Disease

The vast majority of individuals affected by AD are isolated individuals in families, the socalled "sporadic" AD. A small number of families have an inherited form of AD (FAD) for which mutations have been discovered in the APP and presenilin genes[11]. Consequently, from the discovery of mutations and th eir direct relationship with the amyloid physiopathological process, the "*Amyloid cascade hypothesis*" has been established and is driving most of the current research and therapeutic strategies in AD[12,13]. This hypothesis supposes that mutations

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enhance the production of the highly fibrillogenic Aβ peptide 42, that aggregate into oligomers and then plaques. Consequently, Aβ oligomers are supposed to have detrimental neurotoxic and inflammatory side effects, inducing synaptic and neuritic injuries as well as astrocytic and microglial activation. Therefore, the link is made between the amyloidogenesis process and the initial steps towards neurofibrillary degeneration. Although, the research efforts have been inversely proportional to the prevalence of FAD, this hypothesis has to be evaluated in the most represented form of AD, the SAD (sporadic AD).

3. Tau pathology is correlated to AD clinical expression

We have studied tau pathology using a biochemical approach in the human brain from more than 60 non-demented and 70 well-confirmed AD patients[14]. All of those individuals had a clinical examination and the neuropathology was well documented for both amyloid deposits and neurofibrillary tangles. This prospective study has enabled to classify the patient according the distribution of tau pathology in cortical brain areas. This classification is defined by 10 stages that correspond to the sequential and invariable progression of tau pathology in 10 affected brain areas. Thus, tau pathology is first observed in the transentorhinal, the entorhinal and hippocampal formation (Stages S1 to S3). Those brain regions are always affected in individuals aged over 75 years. The neocortical brain regions are affected afterward, starting by the temporal cortex (Stages S4 to S6) and then followed by polymodal association cortical areas (Stages S7 and S8). Until those brain regions are affected, tau pathology can remain clinically silent. Moreover, amyloid deposits are not systematically associated with this tau pathology. This latter observation would suggest that the tau pathology is not necessarily associated with APP pathology and hence, in the absence of any tau mutations or genetic linkage, the pathophysiological mechanisms responsible for the presence and progression of this tau pathology remains unknown. Nonetheless, this phenomenon could be related to normal cerebral aging. However, following this hypothesis, one would expected that older individuals would have more tau pathology than younger. In our series, the tau pathology progression is not closely related to aging, despite the fact that age is definitely a risk factor. The cognitive decline is well correlated with the progression of tau pathology in polymodal association cortical areas and represents the threshold for dementia. Tau pathology then continues its progression towards primary visual and motor cortices (Stages 9 and 10). Our results are in good agreement with neuropathological studies[15-19] and show that tau pathology is following a stereotype progression, knowing the brain anatomy by following cortico-cortical connections; it is invariable and hierarchical, along a network of neurons[14, 20].

4. The APP pathology is correlated to the progression of tau pathology

4.1. Upstream the production of Aβ: the carboxy-terminal fragments of APP

The initiating cause of AD is currently based on the discovery of mutations in APP and presenilin genes. However, no mutations and even polymorphisms on tau, APP or presenilin genes have been associated with SAD[21-27]. The major risk factor is aging, closely followed by ApoE gene polymorphisms[28,29]. Therefore, the initiating events in SAD remain a matter of debate. The APP metabolism has been poorly investigated in SAD. Thus, knowing the precise and detailed progression of tau pathology in the individuals of our brain bank and the correlation with APP expression and its catabolic products upstream Aβ is of major importance. Upstream catabolic products of APP, the so-called carboxy-terminal fragments (CTF) or stubs results from proteases activities named secretases (for review see Suh and Checler, 2002[30]). Three secretases activities have been identified that are the α, β and γ-secretases. The α-secretases cleave APP in the middle of the Aβ sequence (at position 17) and generate the α stub. The CTF precursor for Aβ is resulting from the β-secretase activity, liberating the amino-terminus of Aβ. An alternate cleavage by the β-secretase occurs at position 11 of Aβ leading to β'-stub. APP-CTF β is further processed through γ-secretase producing the Aβ peptide and the cytosolic C51 fragments. For being distinguished from the γ-secretase activity that lead to the production of Aβ, the ε-secretase, which can obviously process all APP-CTFs, liberates the cytosolic domain of APP, also called ε–CTF or C51 (Amyloid precursor protein intracellular domain)[31]. In AD, Aβ-42 deposition strongly suggests that APP metabolisms is altered and APP-CTFs should be modified. We have studied the APP-CTFs expression in both non-demented and AD patients[32]. APP-CTFs were shown to be significantly diminished during the course of AD and moreover, the decrease of APP-CTFs was well correlated with the progression of tau pathology. In addition, the brain tissue of individuals having an inherited form of AD linked to mutations of presenilin 1 also showed a decreased amount of APP-CTFs. Our results thus showed for the first time a relationship between Tau and APP pathologies which is in addition observed early in AD.

4.2. Amyloid deposition is correlated to tau pathology but is lacking the spatiotemporal overlap

The decrease of APP-CTFs should be associated with modification of

the downstream products that are the Aβ peptides. Thus, Aβ peptides were quantitatively and qualitatively investigated, using an extraction in pure formic acid and applied on the brain tissue of all cases from our brain bank. Multiple immunological tools associated with a proteomic analysis were performed. Insoluble Aβ-42 and 40 species were fully solubilized and quantified in the main neocortical areas[20]. The quantities of both species Aβ were compared to the extent of tau pathology, as well as to cognitive impairment. In AD, there is a constellation of amyloid phenotypes, extending from cases with exclusively aggregated Aβ-42 to cases with, in addition, large quantities of insoluble Aβ-40 species. Nonetheless, insoluble Aβ-40 detection was often observed late in the amyloid deposition process (starting at Tau pathology stages 4-5). We observed that there was no obvious spatial and temporal overlap in the distribution of these two insoluble Ab species in cortical brain areas. Thus, brain areas with Aβ-42 was not systematically associated with Aβ-40 deposition and moreover, the quantities of Aβ-40 measured were not directly related to that of Aβ-42. Physical properties were also different. Formic acid-solubilized Aβ-40 aggregates were composed essentially of monomers and dimers, while solubilized Aβ-42 was essentially observed as monomers, dimers, and oligomers. More importantly, Aβ-42 aggregates were observed at the early stages of tau pathology, in non-demented patients. All together, it was interesting to note that during the progression of the disease, Aβ aggregates increase in quantity and heterogeneity, in close parallel to the extension of tau pathology. But unexpectedly, there was no spatial overlap between Aβ aggregation that is widespread and heterogeneously distributed in cortical areas and tau pathology that is progressing sequentially, stereotypically, and hierarchically[20]. Therefore, these observations demonstrate that Aβ42 aggregation, and not Aβ-40, is the marker that is close to Alzheimer aetiology. It should be the main target for the early biological diagnosis of AD and modelling. Furthermore, the spatial mismatch between Aβ and tau pathologies in cortical areas was obvious. First of all, the neocortical areas that contain first or more Aβ are not always the same. Generally, but not always, the occipital cortex is more prone to develop amyloid deposits[20]. These Aβ–42 amyloid deposits observed at the neuropathological level were not related to amyloid angiopathy. Remarkably enough, this brain region is the last to develop tau pathology[14,20]. Conversely, the hippocampal region early affected by tau pathology is not especially affected by amyloid deposition. These observations confirm the findings of Braak and Braak[15,16]. Together, this Aβ/Tau mismatch demonstrates that neurodegeneration is not a direct consequence of extracellular Aβ neurotoxicity (considering that toxicity is mediated through the cell body). Hence, there is a synergetic effect of APP dysfunction on the neuron-to-neuron propagation of tau pathology. This is demonstrated by the fact that tau pathology can be found in the hippocampal area without Aβ deposits[33]. Nonetheless, decrease of APP-CTFs is likely to be more widely distributed over the brain regions, as established in the temporal cortex and occipital, and hence further support the relationship between APP dysfunction and tau pathology[32]. In contrast, the extension of tau pathology in the polymodal association areas is always found in the presence of Aβ deposits, as if these species, directly or indirectly, were necessary to stimulate the progression of tau pathology[33].

4.3. N-truncated Aβ-42 are the seeds for amyloid deposition

Amyloid deposition is the following step to Aβ secretion in AD. The mechanism for aggregation and deposition is uncertain in SAD. Recent observations suggest that aggregation would occur inside the cell body or Aβ would be secreted as oligomers[34-36]. Why these oligomers would develop only in AD is a question that remains to be answered. At the molecular level, Aβ peptide is a heterogeneous catabolic product of APP that varies in length both at the amino- and carboxy-terminus and is also post-translationally modified[37-40]. Thus, Aβ peptide has been shown to be pyroglutamylated, methylated, phosphorylated, oxidized and with isoaspartyl residues[37,41,42]. Therefore, many characteristics could modify the biological properties of Aβ either in aging or in the pathological process. Amino-truncated Aβ peptides has been largely described in fully developed AD and have been suggested to potentiate amyloid fibrillization, amyloid deposition, toxicity and could also anticipate the deposition process in FAD[40,43,44]. Truncation at position 2 to 19, including the β' and α-cleavage sites are found in amyloid deposits of AD individuals and observed on Aβ-42 species[37]. Oxidation occurs on the methionine residue and has been recently suggested to facilitate the release of Aβ from the plasma membrane[45]. For being pathophysiological relevant to AD, truncation or other post-translational modifications should be observed early during the course of AD even before the clinical symptoms. We have adapted two-dimensional gel electrophoresis for the study of aggregates species of Aβ solubilized in pure formic acid[46]. The analysis of Aβ aggregates in fully developed AD individuals demonstrated the accuracy of our approach. Thus, most of truncated Aβ-42 peptides were observed but more importantly, when coupled to western-blotting, two-dimensional gel electrophoresis enable the quantification of each Aβ-42 species. Thus, we demonstrated that more than 60% of all Aβ-42 species were amino-truncated starting from position 2 to 10 and pyroglutamylation at position 3, some of which were oxidized or methylated[46]. We have then used our proteomic approach for the characterization of

Aβ-42 in the very first amyloid deposits observed in preclinical stages of AD. Surprisingly, all of the amino-truncated Aβ-42 were present at the earliest stages of AD and in similar proportion to that observed in fully developed AD. Among all amino-truncated species, particular attention has been paid to the pyroglutamyl variant of Aβ at position 3. This variant has been observed at the earliest steps of amyloid deposition in Down's syndrome and Alzheimer's Disease, as well as in FAD[39,44,47]. It has also been suggested to be more neurotoxic[40]. Other amino-truncated Aβ peptides have been shown to be produced and secreted in cellular models, thus suggesting that truncation is likely not to occur following deposition[48-51]. Altogether, our data strongly suggest that amino-truncated species of Aβ-42 are the seeds for amyloid deposition in SAD. This is also further supported by the fact that in FAD, these pathologically-related variants of Aβ are found to be increased and suggested to potentiate the amyloid deposition process and consequently the anticipation of onset[44]. Besides being characterized since 1985, the importance of modified variants of Aβ have only been poorly investigated in AD and our recent work associated with previous findings highlight the pathophysiological relevance of those variants in AD and their potential usefulness for both diagnostic and therapeutic purposes.

5. AD is a real tauopathy fueled by APP dysfunction

Altogether, our prospective and multidisciplinary study of the natural history of "sporadic" Alzheimer's Disease shows that AD is real tauopathy. This conclusive observation is argued by the fact that without tauopathy, AD cannot be clinically, neuropathologically and molecularly explained. The tauopathy has be present, to progress in cortical areas and to be fueled by multiples parameters to become clinically expressive. Thus, tauopathies relies to all neurodegenerative disorders for which the biological functions of tau are altered. Multiparameters can modify tau functions, starting with tau gene mutations found in frontal-temporal dementia with Parkinsonism link to chromosome 17 until the repeated punches responsible for *dementia pugilistica*[52]. Thus, APP pathology should rather be considered as an additional factor that enhances and potentialises the already existing human tauopathy, thus resulting to the dynamic cortical spreading of the tauopathy that is specific to AD. This hypothesis ce rtainly does not exclude that APP pathology is central to the etiology of AD. In contrast to the "amyloid cascade hypothesis", Aβ-42 by itself is certainly neither the starting point nor the exclusive starting point of AD pathology. Altogether, the loss of APP-CTFs and the early production of amino-truncated Aβ-42 further suggest an early dysfunction of the γ/ε secretase activity. Loss of APP-CTFs has been described in a cellular model[53] and the production of amino-truncated Aβ is observed in increased amounts in FAD with presenilin mutations[44] or in cellular models overexpressing the human APP with mutations close to the γ/ε-secretase cleavage site[51]. Recently, transgenic mouse models supported the hypothesis that APP dysfunction potentiates the tau pathology spreading[53,54]. Conversely, no transgenic mouse models for amyloidosis develop the typical neurofibrillary tangles, definitely showing that Alzheimer's disease result from the association of two degenerative processes that are tau and APP pathologies.

6. Future diagnostic and therapeutic development

6.1. N-truncated Aβ-42 peptides for diagnostic purposes

As demonstrated using our multidisciplinary approach and confirmatory of neuropathologists' observations, appearance of clinical symptoms in AD is already a late event in course of the disease. Many brain areas are affected by amyloid deposits and tau pathology before cognitive impairment. Thus, an early specific and sensitive biological diagnostic is possible and necessary for an early and accurate treatment of AD. The major biological markers at our disposal remain tau protein and Aβ. Many improvements have been given to the use of those markers, as the detection in cerebrospinal fluid of phospho-tau (for review see Blennow and Hampel, 2003[55]). More recently, variants of Aβ peptides have been suggested to improve as well the specificity of AD diagnosis[56,57]. Thus, ratio of carboxy-terminal ending Aβ can distinguish between AD and Creuztfeldt-Jacob disease[58]. Amino-truncated Aβ peptide at position 2 also demonstrated to be elevated in SAD and FAD. Therefore, a better knowledge of Aβ variants related to AD and the development of sensitive tools against those variants would be promising to improve the already existing ELISA or EIA diagnostic kit. Together, the combined use of phospho-tau and N-truncated Aβ-42 peptide should improve dramatically the specificity of the biological diagnosis[55,59,60]. Moreover, such a diagnostic kit would be not only more discriminative toward other neurodegenerative disorders but also necessary as well for the follow-up of treated individuals with cognitive impairment. If such a diagnostic kit is demonstrated to be efficient, it would also circumvent the problem of performing lumbar puncture. Neurologists will certainly adopt this procedure in addition to cognitive assessment if the diagnostic kit is demonstrated to have a high accuracy towards Alzheimer-type degenerating process.

6.2. N-truncated Aβ-42 peptides for therapeutic purposes

Reducing Aβ deposition and/or Aβ production is the main therapeutic goal develop ed in AD. Thus, β-secretase or γ-secretase inhibitors, the proteolytic pathways leading to the

production of Aβ, anti-aggregating molecules (β-sheet breakers), reducing the cholesterol, chelating metals, and vaccination against Aβ, are the main strategies of therapeutic development[30]. The vaccination aiming to clear the brain Aβ deposits has been successful in animal models. The vaccination of transgenic mice producing amyloid plaques by targeting actively or passively the human Aβ leads to a reduced burden of amyloid deposition[61-63] and restore cognitive function[64-66]. In human, vaccination leads to antibodies against amyloid deposits, significantly clear amyloid deposits and reduces the cognitive decline in patients that have developed antibodies against Aβ[67,68]. These results have been obtained on a small cohort of 30 individuals but are, nevertheless, very promising. However, this clinical trial has been stopped because a certain number of vaccinated individuals have developed inflammatory encephalitis, certainly caused by autoimmunity. To overcome this problem of autoimmunity and to make the vaccination a potential preventive and low risk treatment, the antigen selected should be disease-related. One possible perspective is thus to identify Aβ products that are directly related to the earliest stages of amyloid deposition and not found in normal individuals. Amino-truncated Aβ 42 peptides fulfil the definition. To the best of our knowledge, they have not been found in control individuals, and they are observed at the earliest stage of AD and hence, directly related to the pathology. We assume that this specificity has to be confirmed regarding other neurodegenerative disorders, such as dementia with Lewy Body, but amino-truncated Aβ-42 would nonetheless represent outstanding molecular markers for diagnosis and future therapeutic developments. Recent analysis of the immunogenecity of Aβ has shown that the amino-terminal region of Aβ would rather lead to a humoral response, whereas the carboxy-terminal region would generate a T-cell and thus cytotoxic response[69]. Humoral response or passive vaccination using specific antibodies against amino-truncated Aβ would possibly reduce the risk of an autoimmunity response, but it is not excluded that Th2 phenotype of activated T-Cell are also likely to improve the vaccination approach. Altogether, the use of amino-truncated Aβ-42 for the vaccination approach would be triply beneficial by targeting pathologically-related and early amyloidogenic Aβ species and also because the sequences targeted are likely, according to the literature, to generate an humoral response and overall, should reduce the risk of autoimmunity and cytotoxic detrimental side-effects.

Acknowledgements

This work was supported by INSERM and Innogenetics (Ghent, Belgium), IMPRT of Lille and AFRT.

References

1. Alzheimer, A., R.A. Stelzmann, H.N. Schnitzlein, and F.R. Murtagh, An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin Anat*, 1995. 8(6): p. 429-31.
2. Hyman, B.T. and J.Q. Trojanowski, Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol*, 1997. 56(10): p. 1095-7.
3. Glenner, G.G. and C.W. Wong, Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun*, 1984. 120(3): p. 885-90.
4. Kang, J., H.G. Lemaire, A. Unterbeck, J.M. Salbaum, C.L. Masters, K.H. Grzeschik, G. Multhaup, K. Beyreuther, and B. Muller-Hill, The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*, 1987. 325(6106): p. 733-6.
5. Brion, J.P., J. Flament-Durand, and P. Dustin, Alzheimer's disease and tau proteins. *Lancet*, 1986. 2(8515): p. 1098.
6. Delacourte, A. and A. Defossez, Alzheimer's disease: Tau proteins, the promoting factors of microtubule assembly, are major components of paired helical filaments. *J Neurol Sci*, 1986. 76(2-3): p. 173-86.
7. Grundke-Iqbal, I., K. Iqbal, M. Quinlan, Y.C. Tung, M.S. Zaidi, and H.M. Wisniewski, Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem*, 1986. 261(13): p. 6084-9.
8. Ihara, Y., N. Nukina, R. Miura, and M. Ogawara, Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. *J Biochem* (Tokyo), 1986. 99(6): p. 1807-10.
9. Kosik, K.S., C.L. Joachim, and D.J. Selkoe, Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proc Natl Acad Sci U S A*, 1986. 83(11): p. 4044-8.
10. Nukina, N. and Y. Ihara, One of the antigenic determinants of paired helical filaments is related to tau protein. *J Biochem* (Tokyo), 1986. 99(5): p. 1541-4.
11. Marechal, L., D. Campion, and D. Hannequin, [Familial forms of Alzheimer's disease]. *Presse Med*, 2003. 32(16): p. 756-63.
12. Hardy, J. and D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, 2002. 297(5580): p. 353-6.
13. Hardy, J.A. and G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis. *Science*, 1992. 256(5054): p. 184-5.
14. Delacourte, A., J.P. David, N. Sergeant, L. Buee, A. Wattez, P. Vermersch, F. Ghozali, C. Fallet-Bianco, F. Pasquier, F. Lebert, H. Petit, and C. Di Menza, The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology*, 1999. 52(6): p. 1158-65.
15. Braak, H. and E. Braak, Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* (Berl), 1991. 82(4): p. 239-59.
16. Braak, H. and E. Braak, Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging*, 1995. 16(3): p. 271-8; discussion 278-84.
17. Duyckaerts, C., M.A. Colle, F. Dessi, Y. Grignon, F. Piette, and J.J. Hauw, The progression of the lesions in Alzheimer disease: insights from a prospective clinicopathological study. *J Neural Transm Suppl*, 1998. 53: p. 119-26.
18. Duyckaerts, C. and J.J. Hauw, Diagnosis and staging of Alzheimer disease. *Neurobiol Aging*, 1997. 18(4 Suppl): p. S33-42.
19. Mesulam, M.M., Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. *Neuron*, 1999. 24(3): p. 521-9.
20. Delacourte, A., N. Sergeant, D. Champain, A. Wattez, C.A. Maurage, F. Lebert, F. Pasquier, and J.P. David, Nonoverlapping but synergetic tau and APP pathologies in sporadic Alzheimer's disease. *Neurology*, 2002. 59(3): p. 398-407.
21. Roks, G., B. Dermaut, P. Heutink, A. Julliams, H. Backhovens, M. Van de Broeck, S. Serneels, A. Hofman, C. Van Broeckhoven, C.M. van Duijn, and M. Cruts, Mutation screening of the tau gene in patients with early-onset Alzheimer's disease. *Neurosci Lett*, 1999. 277(2): p. 137-9.
22. Delacourte, A. and L. Buee, Tau pathology: a marker of neurodegenerative disorders. *Curr Opin Neurol*, 2000. 13(4): p. 371-6.

23. Crawford, F., M. Freeman, T. Town, D. Fallin, M. Gold, R. Duara, and M. Mullan, No genetic association between polymorphisms in the Tau gene and Alzheimer's disease in clinic or population based samples. *Neurosci Lett*, 1999. 266(3): p. 193-6.
24. Russ, C., J.F. Powell, J. Zhao, M. Baker, M. Hutton, F. Crawford, M. Mullan, G. Roks, M. Cruts, and S. Lovestone, The microtubule associated protein Tau gene and Alzheimer's disease-an association study and meta-analysis. *Neurosci Lett*, 2001. 314(1-2): p. 92-6.
25. Athan, E.S., J.H. Lee, A. Arriaga, R.P. Mayeux, and B. Tycko, Polymorphisms in the promoter of the human APP gene: functional evaluation and allele frequencies in Alzheimer disease. *Arch Neurol*, 2002. 59(11): p. 1793-9.
26. Rocchi, A., S. Pellegrini, G. Siciliano, and L. Murri, Causative and susceptibility genes for Alzheimer's disease: a review. *Brain Res Bull*, 2003. 61(1): p. 1-24.
27. Sorbi, S., B. Nacmias, A. Tedde, P. Forleo, S. Piacentini, S. Latorraca, and L. Amaducci, Presenilin-1 gene intronic polymorphism in sporadic and familial Alzheimer's disease. *Neurosci Lett*, 1997. 222(2): p. 132-4.
28. Lambert, J.C., J. Perez-Tur, M.J. Dupire, D. Galasko, D. Mann, P. Amouyel, J. Hardy, A. Delacourte, and M.C. Chartier-Harlin, Distortion of allelic expression of apolipoprotein E in Alzheimer's disease. *Hum Mol Genet*, 1997. 6(12): p. 2151-4.
29. Lambert, J.C., C. Berr, F. Pasquier, A. Delacourte, B. Frigard, D. Cottel, J. Perez-Tur, V. Mouroux, M. Mohr, D. Cecyre, D. Galasko, C. Lendon, J. Poirier, J. Hardy, D. Mann, P. Amouyel, and M.C. Chartier-Harlin, Pronounced impact of Th1/E47cs mutation compared with -491 AT mutation on neural APOE gene expression and risk of developing Alzheimer's disease. *Hum Mol Genet*, 1998. 7(9): p. 1511-6.
30. Suh, Y.H. and F. Checler, Amyloid precursor protein, presenilins, and alphasynuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. *Pharmacol Rev*, 2002. 54(3): p. 469-525.
31. Chen, F., Y. Gu, H. Hasegawa, X. Ruan, S. Arawaka, P. Fraser, D. Westaway, H. Mount, and P. St George-Hyslop, Presenilin 1 mutations activate gamma 42-secretase but reciprocally inhibit epsilon-secretase cleavage of amyloid precursor protein (APP) and S3-cleavage of notch. *J Biol Chem*, 2002. 277(39): p. 36521-6.
32. Sergeant, N., J.P. David, D. Champain, A. Ghestem, A. Wattez, and A. Delacourte, Progressive decrease of amyloid precursor protein carboxy terminal fragments (APPCTFs), associated with tau pathology stages, in Alzheimer's disease. *J Neurochem*, 2002. 81(4): p. 663-72.
33. Delacourte, A., N. Sergeant, A. Wattez, C.A. Maurage, F. Lebert, F. Pasquier, and J.P. David, Tau aggregation in the hippocampal formation: an ageing or a pathological process? *Exp Gerontol*, 2002. 37(10-11): p. 1291-6.
34. Walsh, D.M., I. Klyubin, J.V. Fadeeva, W.K. Cullen, R. Anwyl, M.S. Wolfe, M.J. Rowan, and D.J. Selkoe, Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*, 2002. 416(6880): p. 535- 9.
35. Wirths, O., G. Multhaup, C. Czech, V. Blanchard, S. Moussaoui, G. Tremp, L. Pradier, K. Beyreuther, and T.A. Bayer, Intraneuronal Abeta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. *Neurosci Lett*, 2001. 306(1-2): p. 116-20.
36. Tabira, T., D.H. Chui, and S. Kuroda, Significance of intracellular Abeta42 accumulation in Alzheimer's disease. *Front Biosci*, 2002. 7: p. a44-9.
37. Kalback, W., M.D. Watson, T.A. Kokjohn, Y.M. Kuo, N. Weiss, D.C. Luehrs, J. Lopez, D. Brune, S.S. Sisodia, M. Staufenbiel, M. Emmerling, and A.E. Roher, APP transgenic mice Tg2576 accumulate Abeta peptides that are distinct from the chemically modified and insoluble peptides deposited in Alzheimer's disease senile plaques. *Biochemistry*, 2002. 41(3): p. 922-8.
38. Masters, C.L., G. Simms, N.A. Weinman, G. Multhaup, B.L. McDonald, and K. Beyreuther, Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A*, 1985. 82(12): p. 4245-9.
39. Saido, T.C., W. Yamao-Harigaya, T. Iwatsubo, and S. Kawashima, Amino- and carboxyl-terminal heterogeneity of beta-amyloid peptides deposited in human brain. *Neurosci Lett*, 1996. 215(3): p. 173-6.
40. Russo, C., E. Violani, S. Salis, V. Venezia, V. Dolcini, G. Damonte, U. Benatti, C. D'Arrigo, E. Patrone, P. Carlo, and G. Schettini, Pyroglutamate-modified amyloid beta-peptides--AbetaN3(pE)--strongly affect cultured neuron and astrocyte survival. *J Neurochem*, 2002. 82(6): p. 1480-9.
41. Roher, A.E., J.D. Lowenson, S. Clarke, C. Wolkow, R. Wang, R.J. Cotter, I.M. Reardon, H.A. Zurcher-Neely, R.L. Heinrikson, M.J. Ball, and et al., Structural alterations in the peptide backbone of beta-amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J Biol Chem*, 1993. 268(5): p. 3072-83.
42. Milton, N.G., Phosphorylation of amyloid-beta at the serine 26 residue by human cdc2 kinase. *Neuroreport*, 2001. 12(17): p. 3839-44.
43. Pike, C.J., M.J. Overman, and C.W. Cotman, Amino-terminal deletions enhance aggregation of beta-amyloid peptides in vitro. *J Biol Chem*, 1995. 270(41): p. 23895-8.
44. Russo, C., G. Schettini, T.C. Saido, C. Hulette, C. Lippa, L. Lannfelt, B. Ghetti, P. Gambetti, M. Tabaton, and J.K. Teller, Presenilin-1 mutations in Alzheimer's disease. *Nature*, 2000. 405(6786): p. 531-2.
45. Barnham, K.J., G.D. Ciccotosto, A.K. Tickler, F.E. Ali, D.G. Smith, N.A. Williamson, Y.H. Lam, D. Carrington, D. Tew, G. Kocak, I. Volitakis, F. Separovic, C.J. Barrow, J.D. Wade, C.L. Masters, R.A. Cherny, C.C. Curtain, A.I. Bush, and R. Cappai, Neurotoxic, redox-competent Alzheimer's β-amyloid is released from lipid membrane by methionine oxidation. *J Biol Chem*, 2003.
46. Sergeant, N., S. Bombois, A. Ghestem, H. Drobecq, V. Kostanjevecki, C. Missiaen, A. Wattez, J.P. David, E. Vanmechelen, C. Sergheraert, and A. Delacourte, Truncated beta-amyloid peptide species in pre-clinical Alzheimer's disease as new targets for the vaccination approach. *J Neurochem*, 2003. 85(6): p. 1581-1591.
47. Saido, T.C., T. Iwatsubo, D.M. Mann, H. Shimada, Y. Ihara, and S. Kawashima, Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. *Neuron*, 1995. 14(2): p. 457-66.
48. Greenfield, J.P., J. Tsai, G.K. Gouras, B. Hai, G. Thinakaran, F. Checler, S.S. Sisodia, P. Greengard, and H. Xu, Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. *Proc Natl Acad Sci U S A*, 1999. 96(2): p. 742-7.
49. Wang, R., D. Sweeney, S.E. Gandy, and S.S. Sisodia, The profile of soluble amyloid beta protein in cultured cell media. Detection and quantification of amyloid beta ein and variants by immunoprecipitation-mass spectrometry. *J Biol Chem*, 1996. 271(50): p. 31894-902.
50. Ancolio, K., C. Dumanchin, H. Barelli, J.M. Warter, A. Brice, D. Campion, T. Frebourg, and F. Checler, Unusual phenotypic alteration of beta amyloid precursor protein (betaAPP) maturation by a new Val-715 Met betaAPP-770 mutation responsible for probable early-onset Alzheimer's disease. *Proc Natl Acad Sci U S A*, 1999. 96(7): p. 4119-24.
51. De Jonghe, C., C. Esselens, S. Kumar-Singh, K. Craessaerts, S. Serneels, F. Checler, W. Annaert, C. Van Broeckhoven, and B. De Strooper, Pathogenic APP mutations near the gamma-secretase cleavage site differentially affect Abeta secretion and APP C-terminal fragment stability. *Hum Mol Genet*, 2001. 10(16): p. 1665-71.
52. Delacourte, A., The molecular parameters of tau pathology. Tau as a killer and a witness. *Adv Exp Med Biol*, 2001. 487: p. 5-19.
53. Gotz, J., F. Chen, J. van Dorpe, and R.M. Nitsch, Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Abeta 42 fibrils. *Science*, 2001. 293(5534): p. 1491-5.
54. Lewis, J., D.W. Dickson, W.L. Lin, L. Chisholm, A. Corral, G. Jones, S.H. Yen, N. Sahara, L. Skipper, D. Yager, C. Eckman, J. Hardy, M. Hutton, and E. McGowan, Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science*, 2001. 293(5534): p. 1487-91.
55. Blennow, K. and H. Hampel, CSF markers for incipient Alzheimer's disease. *Lancet Neurol*, 2003. 2(10): p. 605-13.
56. Wiltfang, J., H. Esselmann, M. Bibl, A. Smirnov, M. Otto, S. Paul, B. Schmidt, H.W. Klafki, M. Maler, T. Dyrks, M. Bienert, M. Beyermann, E. Ruther, and J. Kornhuber, Highly conserved and disease-specific patterns of carboxyterminally truncated Abeta peptides 1-37/38/39 in addition to 1-40/42 in Alzheimer's disease and in patients with chronic neuroinflammation. *J Neurochem*, 2002. 81(3): p. 481-96.

57. Wiltfang, J., H. Esselmann, P. Cupers, M. Neumann, H. Kretzschmar, M. Beyermann, D. Schleuder, H. Jahn, E. Ruther, J. Kornhuber, W. Annaert, B. De Strooper, and P. Saftig, Elevation of beta-amyloid peptide 2-42 in sporadic and familial Alzheimer's disease and its generation in PS1 knockout cells. *J Biol Chem*, 2001. 276(46): p. 42645-57.
58. Wiltfang, J., H. Esselmann, A. Smirnov, M. Bibl, L. Cepek, P. Steinacker, B. Mollenhauer, K. Buerger, H. Hampel, S. Paul, M. Neumann, M. Maler, I. Zerr, J. Kornhuber, H.A. Kretzschmar, S. Poser, and M. Otto, Beta-amyloid peptides in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Ann Neurol*, 2003. 54(2): p. 263-7.
59. Andreasen, N., E. Vanmechelen, H. Vanderstichele, P. Davidsson, and K. Blennow, Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment. *Acta Neurol Scand Suppl*, 2003. 179: p. 47-51.
60. Nagga, K., J. Gottfries, K. Blennow, and J. Marcusson, Cerebrospinal fluid phosphotau, total tau and beta-amyloid(1-42) in the differentiation between Alzheimer's disease and vascular dementia. *Dement Geriatr Cogn Disord*, 2002. 14(4): p. 183-90.
61. DeMattos, R.B., K.R. Bales, D.J. Cummins, J.C. Dodart, S.M. Paul, and D.M. Holtzman, Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*, 2001. 98(15): p. 8850-5.
62. Schenk, D., R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, Z. Liao, I. Lieberburg, R. Motter, L. Mutter, F. Soriano, G. Shopp, N. Vasquez, C. Vandevert, S. Walker, M. Wogulis, T. Yednock, D. Games, and P. Seubert, Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*, 1999. 400(6740): p. 173-7.
63. Schenk, D., P. Seubert, and R.B. Ciccarelli, Immunotherapy with beta-amyloid for Alzheimer's disease: a new frontier. *DNA Cell Biol*, 2001. 20(11): p. 679-81.
64. Janus, C., J. Pearson, J. McLaurin, P.M. Mathews, Y. Jiang, S.D. Schmidt, M.A. Chishti, P. Horne, D. Heslin, J. French, H.T. Mount, R.A. Nixon, M. Mercken, C. Bergeron, P.E. Fraser, P. St George-Hyslop, and D. Westaway, A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature*, 2000. 408(6815): p. 979-82.
65. Janus, C., Vaccines for Alzheimer's disease: how close are we? *CNS Drugs*, 2003. 17(7): p. 457-74.
66. Dodart, J.C., K.R. Bales, and S.M. Paul, Immunotherapy for Alzheimer's disease: will vaccination work? *Trends Mol Med*, 2003. 9(3): p. 85-7.
67. Hock, C., U. Konietzko, A. Papassotiropoulos, A. Wollmer, J. Streffer, R.C. von Rotz, G. Davey, E. Moritz, and R.M. Nitsch, Generation of antibodies specific for betaamyloid by vaccination of patients with Alzheimer disease. *Nat Med*, 2002. 8(11): p. 1270-5.
68. Hock, C., U. Konietzko, J.R. Streffer, J. Tracy, A. Signorell, B. Muller-Tillmanns, U. Lemke, K. Henke, E. Moritz, E. Garcia, M.A. Wollmer, D. Umbricht, D.J. de Quervain, M. Hofmann, A. Maddalena, A. Papassotiropoulos, and R.M. Nitsch, Antibodies against beta-Amyloid Slow Cognitive Decline in Alzheimer's Disease. *Neuron*, 2003. 38(4): p. 547-54.
69. Monsonego, A., V. Zota, A. Karni, J.I. Krieger, A. Bar-Or, G. Bitan, A.E. Budson, R. Sperling, D.J. Selkoe, and H.L. Weiner, Increased T cell reactivity to amyloid beta protein in older humans and patients with Alzheimer disease. *J Clin Invest*, 2003. 112(3): p. 415-22.

The Phenotypic Effects of the apoE Genotype in Transgenic Mice

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Abstract

The isoform E4 of apolipoprotein E (apoE4) is a major risk factor of Alzheimer's Disease (AD) and is associated with the pathology of other neurodegenerative diseases and with increased susceptibility to head trauma. It is not yet known whether the pathological effects associated with the apoE4 genotype are due to the gain of a dominant pathological function by apoE4 or to loss of a neuroprotective function of the other apoE alleles (e.g. apoE3 and apoE2). This question is presently addressed by review of experiments in which mice transgenic for either apoE3 or apoE4 on a null mouse apoE background, and control mice were studied prior to and following expsure to distinct neuronal stress and injury paradigms. This revealed that depending on the experimental paradigm the pathological effects of apoE4 are due either to gain of an isoform specific pathological function (e.g. increased mortality following head injury and diminished neuronal plasticity) or to loss of function (e.g. impaired astrogliosis). These findings suggest that future apoE related therapy may require both anti-apoE4 and apoE3-mimetic approaches each of which may be applicable to different pathological conditions.

Introduction

Apolipoprotein E (apoE), the major brain lipid binding protein, is expressed in humans as three common isoforms termed apoE2, apoE3 and apoE4[1]. A large body of epidemiological and genetic data reveals that apoE4 is strongly linked to both sporadic and late onset familial Alzheimer's Disease (AD) and that apoE4 advances the age of onset of the disease in a gene dosage dependent manner, by as much as 7-9 years[2-4]. ApoE4 is also associated with the pathology of other neurodegenerative diseases such as multiple sclerosis and motor neuron disease[5] and with increased susceptibility to head trauma[6-9]. This suggests that apoE4 impairs general neuronal maintenance and repair mechanisms which are common to many neurodegenerative diseases.

The cellular and molecular mechanisms underlying apoE4 related impairments in neuronal maintenance and repair in AD and in other neurodegenerative disease are not fully understood. Furthermore, it is yet not known whether the pathological effects associated with the apoE4 genotype are due to gain of a dominant pathological feature by apoE4 or to loss of a positive neuroprotective function of the other apoE alleles (e.g. apoE2 and apoE3). The availability of transgenic mice which express distinct human apoE alleles on a null mouse apoE background, and of apoE deficient mice provides powerful model systems for investigating the mechanisms underlying the phenotypic effects of the apoE genotype and for determining the extent to which they are associated with gain of apoE4 specific pathology and/or with loss of a neuroprotective feature of the other apoE alleles. This review addresses these questions and focuses on results of experiments which were performed by our group and which examined the effects of distinct neuronal stress and plasticity paradigms on the phenotype of transgenic mice expressing human regulatory and coding sequences of either apoE3 or apoE4 on a null mouse apoE background.

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Susceptibility to head injury: apoE3 is neuroprotective whereas apoE4 increases fatalities

The apoE4 genotype in man is associated with increased susceptibility to brain injury[6-9]. We thus examined the extent to which this effect can be mimicked in head injured apoE transgenic mice and whether related features such as fatality and recovery from injury are affected differentially by the apoE genotype[9]. This revealed that more than 50 percent of the apoE4 mice die following closed head injury whereas only half as many of the transgenic mice expressing apoE3 and of the matched control and apoE deficient mice die after being exposed to the same head injury (Figure 1A). Clinical assessment of the surviving mice[9] revealed that all mice groups displayed similar severity of neurological damage immediately following injury. In contrast, the extent of neurological impairments of the apoE3 transgenic mice on subsequent days was significantly lower than those of both the apoE4 transgenic and the apoE deficient mice whose scores were similar (Figure 1B). This suggests that the neurological recovery of the apoE3 transgenic mice is better than those of the other mice groups.

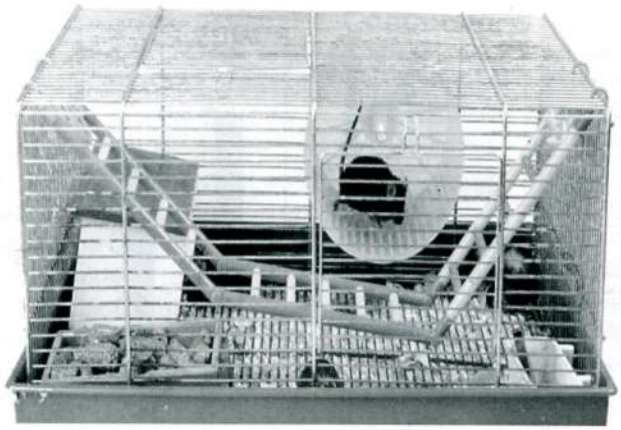

Figure 2. An enriched environment cage.

Taken together these findings show that apoE4 transgenic mice are more susceptible to closed head injury than apoE3 transgenic mice and that this effect is due to both a protective effect of apoE3 and to apoE4 related increased mortality. Further analysis of these results reveals that the apoE4 dependent increase in fatality following closed head injury is associated with gain of a pathological function (e.g. the mortality rank order is apoE4 > apoE3 ~ control ~ apoE deficient), and that the improved recovery of the apoE3 transgenic mice is associated with gain of a neuroprotective function (e.g. the recovery rank order is apoE3 > ApoE4 ~ control ~ apoE deficient).

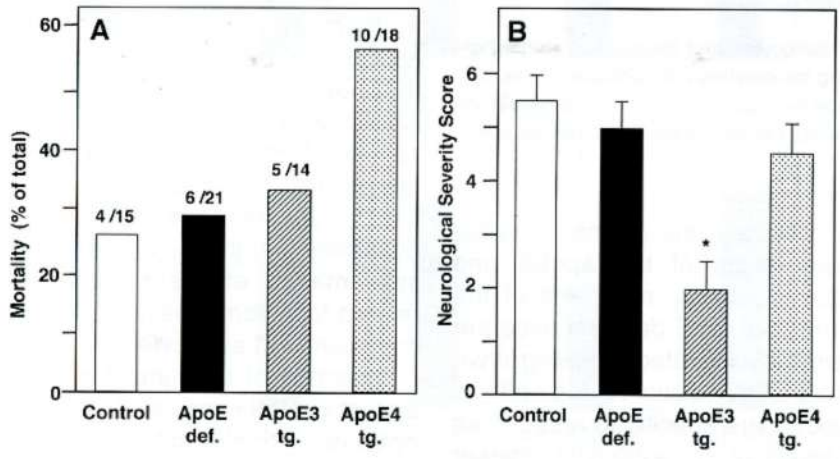

Figure 1. The effects of closed head injury on the mortality (A) and neurological recovery (B) of apoE3 and apoE4 transgenic mice and of apoE deficient and control mice. Results correspond to the number of mice out of the total of each group which died between 24 h and 11 days after closed head injury (A) and to the recovery of the surviving mice (B) as assessed on a neurological severity scores scale (0 = non injured mice) at 12 days following injury.

The effects of apoE genotype on hippocampal plasticity

Exposure of mice to an enriched environment which consists of social interactions and toys, such as tunnels and a running wheel (Figure 2), stimulates exploratory and motor behavior and has pronounced plastic consequences. These include activation of synaptogenesis and improvements in learning and memory[10]. Accordingly the phenotypic effects of the different apoE genotypes were also assessed by measurements of the effects of the apoE genotype on the neuronal and cognitive plastic responses elicited by environmental stimulation. T-maze measurements of learning and memory revealed that the learning ability of control and apoE3 transgenic mice increases by exposure to the enriched environment whereas that of the apoE4 mice is unaltered[11]. Furthermore the working memory of the control and apoE3 mice improves markedly following environmental stimulation whereas that of the apoE4 transgenic mice is not affected by the environmental conditions and is similar to that of the non stimulated apoE3 transgenic and control mice[11]. The apoE deficient mice have swimming stamina problems and were thus not subjected to this T-test[11]. The extent to which the cognitive deficits of the apoE4 transgenic mice are related to

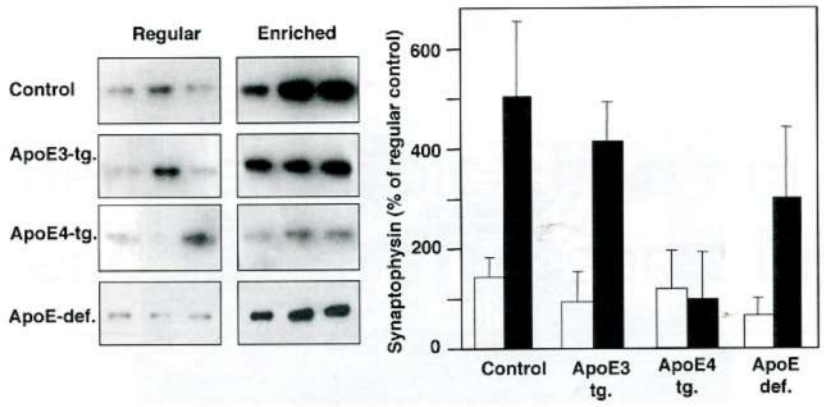

Figure 3. The effects of environmental stimulation on the hippocampal levels of the presynaptic protein synaptophysin of apoE3 (ApoE3-tg) apoE4 (ApoE4-tg) transgenic mice and of apoE deficient (ApoE-def.) and control mice. Results shown on the left are representative immunoblots of three mice in each of the groups whose quantification for 4-5 mice per group is shown on the right.

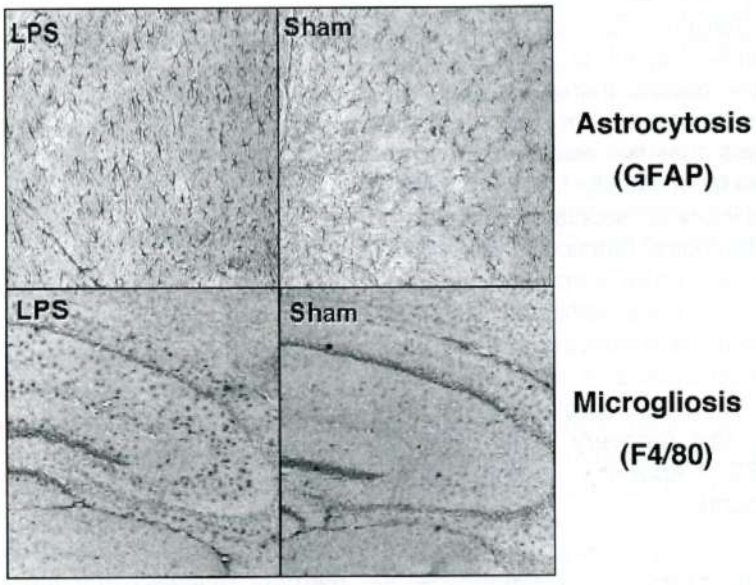

Figure 4. LPS mediated activation of hippocampal astrocytes and microglia. Coronal hippocampal sections prepared from mice 3 days following icv injection with LPS and from sham treated mice were stained with either anti GFAP mAbs (astrocytes) or anti F4/80 antiserum (activated microglia). The cell nuclei of the F4/80 stained sections were visualized by hemitoxylen staining.

impairments in synaptic plasticity was investigated by immunoblot measurements of the levels of the presynaptic protein synaptophysin[12]. This revealed that environmental stimulation induces a pronounced increase in hippocampal synaptophysin levels of the apoE3 transgenic, control and apoE deficient mice whereas those of the apoE4 transgenic mice are unaffected by this treatment (Figure 3).

In contrast, the cortical synaptophysin levels of the apoE3 and apoE4 transgenic mice and of the control and apoE deficient mice are all similarly elevated following environmental stimulation. These effects of apoE4 are specific to neurons as the levels of the astrocytic marker glial acidic fibrillary protein (GFAP) are not affected either by the apoE genotype or by environmental stimulation.

Furthermore, NGF whose levels are elevated by environmental stimulation[10], has apoE genotype and brain area dependencies, similar to those seen with synaptophysin[11] both prior to and following environmental stimulation.

Further examination of the cognitive and neuronal effects of apoE reveals that the rank order of the environmentally induced synaptogenesis and improvements in learning and memory in the different mice groups is apoE4-tg. << apoE3-tg. ~ control ~ apoE def. This suggests that the impaired response of the apoE4 transgenic mice in the enriched environment paradigm is due to gain of an apoE4 dependent pathological function which reduces neuronal plasticity.

These animal model findings are in accordance with the observation that the apoE4 genotype in AD is associated with impaired plasticity[13] and that education in early life lowers the risk of AD in apoE3 but not in apoE4 subjects[13-17].

Isoform specific effects of apoE on astrogliosis

AD is associated with chronic brain inflammation which includes activated microglia and astrocytes and elevated levels of distinct inflammatory proteins such as 1-antichymotrypsin and the complement system[18-20]. Furthermore brain inflammation and astrocyte activation are more robust in apoE4 positive AD patients[21]. ApoE, which is synthesized and secreted from astrocytes, can modulate microglial and astrocyte activation[22-24]. We therefore examined the possibility that the genotype specific inflammatory effects of apoE are related to isoform specific effects on microglial and astrocyte activation.

Injection of LPS into the ventricles of control mice results in astrocyte and microglial activation[25] which can be visualized immunohistochemically with anti GFAP and anti F4/80 mAbs (Figure 4). The microglial and astrocytic response to

LPS of control mice decreases progressively with age[25], and the effects of apoE were therefore monitored at two age groups. LPS treatment of 6 months old apoE transgenic and control mice activates brain astrocytes of control and apoE3 transgenic mice but has no effect on astrogliosis of age matched apoE deficient and apoE transgenic mice (Figure 5).

The opposite effect is seen with 12 months old mice where LPS, under similar conditions (e.g. stimulation for 72 hours), activates brain astrocytes of apoE deficient and apoE4 transgenic mice but not of control and apoE3 transgenic mice. LPS also induces microglial activation which however is unaffected by either apoE deficiency or apoE genotype. The age dependency of the effects of apoE on astrogliosis seems to be due to kinetic differences. Accordingly in 6 months old control and apoE3 transgenic mice LPS produces a response which increases progressively for up to at least 3 days whereas the corresponding response of the apoE4 and control mice is too slow and thus not detectable[25]. At 12 months the overall LPS response of all groups is faster such that with the control and apoE3 mice it is transient and returns to base line by 3 days whereas the corresponding response of the apoE4 and apoE deficient mice now yield a progressive response which at 3 days results in a marked LPS specific effect.

The mechanisms underlying the age dependency of the effects of apoE on the kinetics of LPS induced astrogliosis are not known. However, the results clearly show that apoE plays a regulatory role in the activation of brain astrocytes and that this effect is mimicked by human apoE3 but not by apoE4 which yields a phenotype similar to that of apoE deficient mice. Thus, unlike in the head injury and enriched environments paradigms the effects of apoE4 on astrocytes activation are associated with a loss of function.

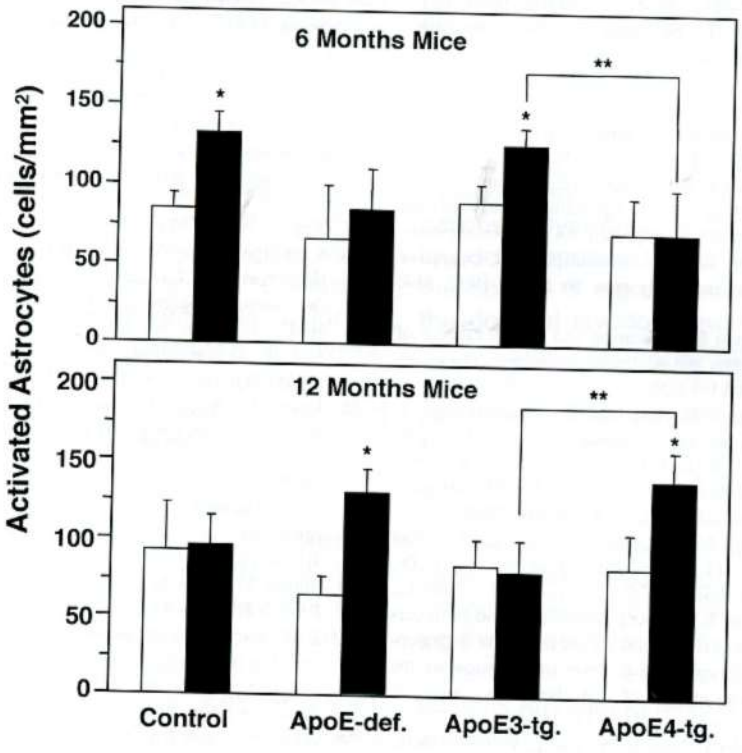

Figure 5. LPS mediated activation of hippocampal astrocytes in brains of 6 and 12 months old apoE3 transgenic (ApoE3-tg) and apoE4 (ApoE4-tg) transgenic mice and apoE-deficient (ApoE-def) and control mice. Astrocytes were visualized immunohistochemically with an anti GFAP mAb and the results shown are the number of activated astrocytes/mm².

Conclusions

The phenotypic expressions of the apoE genotype are a function of the experimental paradigm with which the mice are being challenged. Accordingly diminished neuronal plasticity of apoE4 transgenic mice following environmental stimulation and the increased mortality of these mice following head injury are both due to gain of pathological functions. In contrast the isoform specificity of the effects of apoE on astrogliosis are due to a loss of function of apoE4. These conclusions are based on comparisons of the respective phenotypes of apoE3, apoE4 transgenic mice and apoE deficient mice and less so on the corresponding phenotypes of control mice, which in the enriched environment, head injury mortality and LPS paradigms, are similar to those of the apoE3 transgenic mice. However, the gain of improved repair of the apoE3 transgenic mice following head injury reflects differences between mouse apoE and human apoE3. This phenotype may thus be specific to the mouse model. The results reviewed thus far were obtained with transgenic mice whose apoE3 and apoE4 expression is regulated by human regulatory sequence[26]. Parallel studies with apoE transgenic mice whose apoE is expressed either with an astrocytic or a neuron specific promoter revealed that, depending on the experimental paradigm, the phenotypic expression of apoE4 in these mice is also either loss of function or gain of pathological function by apoE4[27-30].

Application of the apoE transgenic model findings to man suggests that the effects of apoE4 in AD and other diseases may be mediated by gain as well as loss of functions. This has important therapeutic ramifications, since future treatment of diseases whose pathology is related to gain of a pathological function of apoE4 should be based on an "anti apoE4" approach whereas treatment of diseases whose apoE4 related pathology is associ-

ated with loss of function will require apoE3 mimic approaches. An additional level of diversity and complexity is the molecular mechanisms which mediate the phenotypic effects of apoE4. These include isoform specific effects of apoE on APP and amyloidogenesis, as well as on brain lipid metabolism and neuronal function[1]. Future studies of the role of these mechanisms in the expression of distinct phenotypic features of the different apoE genotypes are needed for unraveling their distinct role in specific diseases and for the design of the appropriate therapeutic approaches.

Acknowledgements

This work was supported partly by grants to DMM from the European Community (Grant # 2001/00972), from the Fund for Basic Research sponsored by the Israel Academy of Sciences and Humanities (Grant # 43/00-1), from the Harry Stern National Center of Alzheimer's Disease and Related Disorders, from the Eichenbaum Foundation. DMM is the incumbent of the Myriam Lebach Chair in Molecular Neurodegeneration. Figures 2 and 5 were adapted from references 11 and 25 with the permission of Elsevier.

References

1. Mahley R.W., Rall S.C., Apolipoprotein E: far more than a lipid transport protein. *Ann Rev Genom Hum Genet* 2001; 1: 505-37.
2. Corder E.H., Saunders A.M., Strittmatter W.J., Schmechel D.E., Gaskell P.C., Small G.W., Roses A.D., Haines J.L., Pericak-Vance M.A., Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; 261: 921-3.
3. Saunders A.M., Strittmatter W.J., Schmechel D., George-Hyslop P.H., Pericak-Vance M.A., Joo S.H., Rosi B.L., Gusella J.F., Crapper-MacLachlan D.R., Alberts M.J., et al., Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993; 43: 1467-72.
4. Roses A.D., Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Ann. Rev Med* 1996; 47: 378-400.
5. Chapman J., Korczyn A.D., Karussis K., Michaelson D.M., The effects of APOE genotype on age at onset and progression of neurodegenerative diseases. *Neurology* 2001; 57: 1482-5.
6. Roses A.D., Saunders A.M., Head injury, amyloid beta and Alzheimer's disease. *Nat Med* 1995; 1: 603-4.
7. Sorbi S., Nacmias B., Piacentini S., Apolipoprotein E genotypes and outcome after post-traumatic coma. *Neurology* 1996; 46: A307.
8. Lichtman S.W., Seliger G., Tycko ., Marder K., Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. *Neurology* 2000; 55(10): 1536-9.
9. Sabo T., Lomnitski L., Nyska A., Beri S., Maronpot R.R., Shohami E., Roses A.D., Michaelson, D.M., Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: the allele E3 is neuroprotective whereas E4 increases fatalities. *Neurosciences* 2000; 101: 879-84.
10. Van Praag H., Kempermann G., Gage F.H., Neural consequences of environmental enrichment. *Nat Rev Neurosci* 2000; 1: 191-8.
11. Levi O, Jongen-Relo A.L., Feldon J., Roses A.D., Michaelson D.M., ApoE4 impairs hippocampal plasticity isoform-specifically and blocks the environmental stimulation of synaptogenesis and memory. *Neurobiol Dis* 2002; 13: 273-82.
12. Saito S., Kobayashi Y., Igarashi M., Komiya Y., Ando S., Decreased synaptic density in aged brains and it's prevention by rearing under enriched environment as revealed by synsptophysin contents. *J Neurosci Res* 1994; 39: 57-62
13. Arendt T., Schindler C., Bruckner M.K., Eschrich K., Bigl V., Zedlick D., Marcova, L., Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein E4 allele. *J Neurosci* 1997; 17: 516-29.
14. Kalmijin S., Feskens E.J., Launer L.J., Kromhout D., Longitudinal study of the effects of apolipoprotein E4 allele on the association between education and cognitive decline in elderly men. *Br Med J* 1997; 314: 34-5.
15. Katzman R., Education and the prevalence of dementia and Alzheimer's disease. *Neurology* 1993; 43: 13-20.
16. Kawas C.H., Katzman R., Epidemiology of dementia and Alzheimer's disease. In: Terry R.D., Bick K.L. and Sisodia S.S., eds., .Alzheimer's Disease 2nd ed., Philadelphia: *Lippincott, Williams & Wilkins*, 1999.
17. Moceri V.M., Kukull W.A., Emanuel I., Van Belle G., Starr J.R., Schellenberg G.D., McCormick W.C., Bowen J.D., Teri L., Larson E.B., Using census data and birth certificates to reconstruct the early life socioeconomic environment and the relation to the development of Alzheimer's disease. *Epidemiology* 2001; 12: 383-9.
18. Rogers J., Webster S., Lue L. F., Civin W.H., Emmerling M., Shivers B., Walker D., McGeer P., Inflammation and alzheomer's diseasepathogenesis. Neurobiol. *Aging* 1996; 17: 681-6.
19. McGeer P.L., McGeer E.G., Inflammation of the brain in Alzheimer's disease: implications for therapy. *J Leukoc Biol* 1999; 65(4): 409-15.
20. Halliday G., Robinson S.R., Shepherd C., Kril J., Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. *Clin Exp Pharmacol Physiol* 2000; 27(1-2): 1-8.
21. Licastro F., Mallory M., Hansan L.A., Masliah E., Increased levels of alpha-1-antichymotrypsin in brains of patients with Alzheimer's disease correlate with activated astrocytes and are affected by apoE4 genotype. *J Neuroimmunol* 1998; 88: 105-10.
22. Vitek M., Snell J., Dawson H., Colton. C.A., Modulation of nitric oxide production in human macrophages by apolipoprotein-E and amyloid–betapeptide. *Biochem Biophys Acta* 1997; 240: 391-4
23. Laskowitz D.T., Matthew W.D., Bennett E.R., Schmechel D., Herbstreith M.H., Goel S., McMillian M.K., Endogenous apolipoprotein E suppresses LPS-stimulated microglial nitric oxide production. *Neuroreport* 1998; 9(4): 615-8.
24. Muller W., Meske V., Berlin K., Scharnagel H., Marz W., Ohm T.G., ApolipoproteinE isoforms increase intracellular Ca+2 differentially through an omega-agatoxin sensetive Ca+2 channel. *Brain Pahtol* 1998; 8: 641-53.
25. Ophir G., Meilin S., Efrati M., Chapman J., Karussis R., Roses A., Michaelson D.M., Human apoE3 but not apoE4 increases impaired astrocyte activation in apoE null mice. *Neurobiol Dis* 2003; 12: 56-64.
26. Xu P.T., Schmechel D., Rothrocl-Christian T., Burjharth D.S., lin H.L., Popko B., Sullivan P., Maeda N., Saunders A.M., Roses A.D., Gilbert J.R., Human apolipoprotein E2, E3 and E4 isoform specific transgenic mice: human like-patterns of glial and neuronal immunoreactivity in central nervous system not observed in wild-type. *Neurol Dis* 1996; 3: 229-45.
27. Fagan A.M., Murphy B.A., Patel S.N., Kilbridge J.F., Mobley W.C., Bu G., Holtzman D.M., Evidence for normal aging of the septo-hipocampal cholinergic system apoE (-/-) mice but impaired clearance of axonal degeneration products following injury. *Exp neurol* 1998; 151: 314-25.
28. Raber J., Wong D., Buttini M., Orth M., Bellosta S., Pitas R.E., Mahley R.W., Mucke L., Isoform specific effects of human apolipoprotienE on brain function revealedin apoE knockout mice: increased susceptibility offemales. Proc Nat. *Acad Sci* 1998; 96: 10914-19.
29. Buttini M., Orth M., Bellosta S., Akeefe H., Pitas R.E., Wyss-Corcy T., Mucke L, Mahley R.W., Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: isoform-specific effects on neurodegeneration. *J Neurosci* 1999; 19: 4867-80.
30. White F., Nicoll J.A., Roses A.D., Horsburgh, K., Impaired neuronal plasticity in transgenic mice expressing human apolipoprotein E4 compared to E3 in a model of entorhinal cortex lesion. *Neurobiol Dis* 2001; 8: 611-25.

Memantine Treatment of three Parkinsonian Patients Having Dyskinesia and Cognitive Decline

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Background

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disease characterised by degeneration of basal ganglia dopaminergic neurons[1], leading to the characteristic clinical motor symptoms of tremor, hypokinesia, rigidity and postural dysfunction[2]. Modern dopamine enhancing therapies with levodopa and dopamine agonists can compensate for the dopamine deficits resulting in good symptomatic improvement during early years of treatment[3]. In best cases, patients can have a complete relief of symptoms only to develop motor complications after 5 - 10 years of levodopa treatment. After a further 5 - 10 years patients may develop neuropsychiatric symptoms with nightmares, hallucinations, impaired cognition and finally end up in a palliative, treatment refractive phase with pronounced symptoms[4].

Motor complications present themselves as insufficient and reduced effects of medication, sudden shifts between over- and underfunction of movements (on-off phenomenon), as well as motor stops or involuntary movements, dyskinesia[5,6]. Dyskinesia is the most common, unwanted effect of chronic levodopa treatment and presents a therapeutic dilemma as the therapeutic window becomes smaller with time. In a review of 74 publications with adequate data related to frequency of levodopa-induced dyskinesias, Ahlskog and Muenter reported that after 5 years of levodopa therapy about 40% of Parkinsonian patients experience dyskinesias[7]. The way in which dopamine receptors are stimulated by levodopa seems to be a key factor in the development of motor complications[8]. Dyskinesia induction with levodopa appears to be related to the degree of nigral denervation, to the dose of levodopa employed, to the frequency of drug administration, and to brain exposure to levodopa[9]. However, this is probably not only due to the metabolism of levodopa but also to a dysfunction of peripheral absorption of levodopa.

The concentration limits of antiparkinsonian medication become narrower over time, thereby increasing the tendency for motor over- and underfunction[10]. Levodopa treated patients are said to have a 10% risk per year of motor fluctuations[11]. In addition, non-motor complications such as sleep disturbance, hallucinations, depression or cognitive failure often appear during the course of the pharmacologically treated disease[12].

The treatment of dyskinesia is both a problem and challenge to the physician, whose major aim is to obtain as constant dopamine concentration as possible to avoid a pulsatile action on the dopamine receptors[13]. This can be achieved with levodopa in a number of ways, including multiple fractioned dosing, administration of depot preparations, addition of enzyme inhibitors (COMT and MAO-B) or continuous duodenal infusion[14]. However, dyskinesia may persist despite these strategies and even with complementary treatment with dopamine agonists[15].

It has been postulated that the presence of an overactive glutamatergic neurotransmission in the basal ganglia may be important to the development of dyskinesias[16]. The pulsatile or abnormal stimulation of dopamine receptors by short-acting dopaminergic agents is also believed to indirectly affect NMDA receptors resulting in them having an increased sensitivity to activation

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by glutamate. These interactions could clinically manifest themselves as dyskinesia[17]. Followingly, this provides a rationale for administrating the NMDA receptor antagonist amantadine as a potential, although often under-used, therapeutic possibility[18]. Amantadine has been used for a long time as anti-parkinsonian treatment and can also suppresss established levodopa-induced dyskinesia suggesting a link between glutamatergic systems and the process that underlies the genesis of involuntary movements[19,20].

Memantine is another interesting NMDA receptor antagonist. Memantine has recently been launched in Sweden for the treatment of moderate to severe Alzheimer's Disease[21]. A number of randomised, placebo-controlled, double blind studies have indicated that memantine is efficacious in moderate and severe Alzheimer's disease[22,23]. Memantine was reported to significantly improve global functioning as well as cognitive ability. Only few small studies have been performed with memantine on idiopathic Parkinson patients, however with no record of cognitive decline[24,25]. Parkinsonian features improved in half of the patients (n=10) with memantine in one study[24] and significantly drug effect in both on and off states in the other study (n=12)[25]. However, there were no anti-dyskinetic effects observed.

The pharmacological profile of memantine is similar to that of amantidine with a non-competitive NMDA receptor antagonism with moderate affinity. It blocks the effect of pathologically increased tonic glutamate levels that would otherwise lead to neuronal dysfunction[26]. Dysfunction of glutamatergic neurotransmission via NMDA receptors contributes to the dyskinetic symptomatology associated with Parkinson's disease and to the appearance of symptoms and disease progression of neurodegenerative dementias[16, 21, 27].

Considering this theoretical basis[27], we tried memantine as a complementary medication in three dyskinetic, cognitively impaired Parkinsonian patients in order to evaluate any possible anti-dyskinetic drug effect besides from the effects on cognition. Patients were evaluated in an outpatient setting while they were on. The severity of Parkinsonian symptoms was measured by the Hoehn & Yahr scale[28] and the cognitive status through MMT (Mini Mental Test)[29]. Dyskinesia (DK) was evaluated with a scoring on a 5-grade scale ranging from 0 (no DK) to 4 (severe DK) similar to that of the UPDRS part IV; 33 (Unified Parkinson´s Disease Rating Scale)[30]. None of the patients had been exposed to neuroleptic medication for the last six months.

Case descriptions

Case 1

A 79-year-old woman, living alone, who has had Parkinson's disease for the last 12 years of which the last 9 with levodopa therapy, now in Hoehn-Yahr IV. The patient had dyskinesia for the 4 last years that did not improve neither by selegiline addition, fractioned dosing of levodopa (150 mg x 6) nor with addition of COMT-inhibitor (6 times a day). Two different dopamine agonists (pramipexole and kabergoline) were also tried without anti-dyskinetic effects also showing side effects of dizziness and nausea.

The patient received home help three times a day and exhibited a MMT score of 18/30. Reducing the dose of L-dopa was tested but this resulted in increased Parkinsonian symptoms. Therefore, the patient was returned to the original dosing schedule.

Memantine 10 mg x 1 for 6 weeks had no effect on memory tests, ADL-function or dyskinesia. A doubling of the dose resulted in improvements of the dyskinesia (2/4 to 1/4) and cognition (20/30) after one month, however no effect on Hoehn & Yahr.

Case 2

An 82-year-old man with 5 years of Parkinson's disease, Hoehn-Yahr III, and dyskinesia for the last 2 years. The patient is currently treated with 800 mg levodopa 8 times a day accompanied with COMT-inhibition with every levodopa dosage as well as the dopamine agonist pramixipexole (1 mg x 3). The patient lives together with his wife in own housing and does not receive home help. The patient had a memory testing in MMT score of 20/30.

He received memantine 10 mg x 1 for one month with no effect on MMT scores but an anti-dyskinetic effect (3/4 to 2/4). The subsequent increased dose of 10 mg x 2 gave a further improvement of the dyskinesia (2/4 to 1/4) as well as in Hoehn & Yahr (3 to 2,5) after another month but still no effect on cognition.

Case 3

A 75-year-old woman with an 8 year history of Parkinson's disease, now in Hoehn & Yahr IV, and dyskinesia (3/4) during the last year. She also had Alzheimer dementia with a MMT score of 14/30 and was receiving a nightly 10 mg dose of the cholinesterase inhibitor donepezil for the impaired cognition[31]. She was receiving depot tablets of levodopa 4 times a day in combination with rapidly dissolving 50 mg tablets of levodopa likewise 4 times a day. An attempted dose reduction resulted in increased Parkinsonian symptoms. COMT-inhibition was tried but discontinued since it resulted in increased dyskinesias that remained even after an attempt of levodopa reduction. The dopamine agonist pramipexole resulted in a reduction of blood pressure as well as hallucinations. Amantadine (100 mg x 2) improved dyskinesia (3/4 to 2/4), however along with side effects of anxiety and hallucinations and was thus discontinued.

Memantine 10 mg every evening resulted in insomnia, whereas the same dose instead given every

morning had no side effects, however with no anti-dyskinetic effect after 5 weeks. A dose increase of memantine to 10 mg x 2 had a general activating effect as well as an increased MMT score (14/30 to 16/30) following 3 months of treatment, however still with no anti-dyskinetic or anti-Parkinson effect.

Discussion

Two of these three patients provide examples of an anti-dyskinetic effect of memantine on cognitively impaired Parkinsonian patients in addition to the original, approved indication of moderate to severe Alzheimer's Disease. As the anti-dyskinetic and positive cognitive effects were so favourable it was considered unethical and malpractice to discontinue the medications in order to establish whether the effects thereby would cease, thus strengthening causation.

The theoretical pharmacological basis of an NMDA receptor antagonist affecting the glutamatergic function and not the dysfunctional dopamine system appears to have been reflected in a clinical anti-dyskinetic effect in two of these three cases.

Randomised, controlled, double blind studies with this type of NMDA receptor antagonist memantine / placebo should be encouraged to be performed on dyskinetic, cognitively impaired Parkinsonian patients with pharmacological difficulties to overcome their motor dysfunction. As dyskinesia is one of the major Parkinsonian problems, any remedy that could alleviate this symptomatology would be of great value to both the patient and the doctor.

References

1. Agid Y. Dopaminergic systems in Parkinson´s disease. In: Quinnn N and Jenner P(Eds). *Disorders of movement: clinical, pharmacological and physiological aspects*. London: Academic Press, 1989: 85-107
2. Marsden CD. Parkinson`s disease. *J Neurol Neurosurg Psychiatry* 1994;57:672-81
3. Calne DB. Treatment of Parkinson´s disease. *N Engl J Med* 1993;329:1021-27
4. Jankovic J. Pathophysiology and clinical assessment of motor symptoms in *Parkinson´s disease*. In: Koller W (Ed.). Handbook of Parkinson´s Disease (pp 99-126). New York: Marcel Dekker Inc.
5. Mouradian MM et al. Motor fluctuations in Parkinson´s disease: pathogenetic and therapeutic studies. *Ann Neurol* 1987;22:475-79
6. Martignoni E et al. Motor complications in Parkinson´s disease. *Neurol Sci* 2003;24(Suppl 1):S27-9
7. Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulated literature. *Mov Disord* 2001;16:448-58
8. Agid Y, Chase T, Marsden D. Adverse reactions to levodopa: drug toxicity or progression of the disease? Lancet 1998;351:851-52
9. Pearce RKB, Heikkila M, Lindén I-B et al. L-dopa induces dyskinesia in normal monkeys: behavioural and pharmacokinetic observations. *Psychopharmacology* 2001;156: 402-09
10. Olanow CW, Obeso JA. Pulsatile stimulation of dopamine receptors and levodopa-induced motor complications in Parkinson´s disease: implications for the early use of COMT inhibitors. *Neurology* 2000;55(Suppl 4):S72-S77
11. Van Laar T. Levodopa-induced response fluctuations in patients with Parkinson´s disease: strategies for management. *CNS Drugs* 2003;17:475-89
12. Witjas T et al. Nonmotor fluctuations in Parkinson´s disease: frequent and disabling. Neurology 2002;59:408-13
13. Chase TN. The significance of continuous dopaminergic stimulation in the treatment of Parkinson´s disease. *Drugs* 1998;55(Suppl 1):1-9
14. Nyholm D. Pharmacotherapy for Parkinson´s Disease – Observations and Innovations. Academic thesis, Uppsala University, *Acta Universitatis Upsaliensis*, Sweden, 2003
15. Marsden CD. Clinical experience with cabergoline in patients with advanced Parkinson´s disease treated with levodopa. *Drugs* 1998;55(Suppl 1):17-22
16. Merims D et al. The role of glutamatergic transmission in the pathogenesis of levodopa-induced dyskinesias. Potential therapeutic approach. *Neurol Neurochir Pol*. 2001;35 (Suppl 3):65-8
17. Verhagen Metman L et al. Pathophysiology of motor response complications in Parkinson´s disease: hypotheses on the why, where, and what. *Mov Disord* 2000;15:3-8
18. Blanchet PJ et al. Renaissance of amantadine in the treatment of Parkinson´s disease. *Adv Neurol* 2003;91:251-57
19. Del Dotto P et al. Intravenous amantadine improves levadopa-induced dyskinesias: an acute double-blind placebo-controlled study. *Mov Disord* 2001;56:1239-42
20. Paci C et al. Amantadine for dyskinesia in patients affected by severe Parkinson´s disease. *Neurol Sci* 2001;22:75-6
21. Jarvis B, Figgitt D. Memantine. *Drugs Aging* 2003;20:465-76
22. Winblad B et al. Memantine in severe dementia: results of the 9M-Best Study. Benefit and Efficacy in severely demented patients during treatment with memantine. *Int J Geriatr Psychiatr*. 1999;14:135-46
23. Reisberg B et al. Memantine in moderate to severe Alzheimer´s disease. *N Engl J Med* 2003;348:1333-41
24. Rabey JM, Nissipeanu P, Korczyn AD. Effcacy of memantine, an NMDA receptor antagonist, in the treatment of Parkinson´s disease. *J Neural Transm* 1992;4:277-82
25. Merello M, Nouzeilles MI, Cammarota A, Leiguarda R. Effect of Memantine (NMDA antagonist) on Parkinson´s disease: A double-blind crossover randomized study. *Clin Neuropharmacology* 1999;22(3): 273-76
26. FASS (Pharmacological Specialities in Sweden). 2003; pp 518-19. Elanders, Kungsbacka, Sweden
27. Peeters M et al. Distinct effects of amantadine and memantine on doapminergic transmission in the rat striatum. *Neurosci Lett* 2003;343:205-9
28. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967;17:427-42
29. Folstein MF et al "Mini-Mental-State". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-98
30. Martinez-Martin P, Gil-Nagel A, Gracia LM et al. Unified Parkinson´s Disease Rating Scale characteristics and structure. The Cooperative Multicentric Group. *Mov Disord* 1994;9:76-83
31. FASS (Pharmacological Specialities in Sweden). 2003; pp 181-82. Elanders, Kungsbacka, Sweden

Prevalence of Co-Morbidities in Mild Cognitive Impairment (MCI) Patients

A Romanian Descriptive Study

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Abstract

Mild Cognitive Impairment (MCI) is a risk factor for developing AD; it is believed that medical intervention in individuals with MCI can help delay the onset of AD. It is important to understand that a diagnosis of MCI does not mean that an individual will develop AD. Rather, individuals with MCI, if left untreated, are at increased risk to develop AD at a rate of 12-15 % per year, compared with just 1% per year for healthy people over the age of 65. Early detection of cognitive impairment is very important for people because these are medical conditions that are reversible under appropriate therapy such as vitamin deficiencies, depression and hypotiroidism. Those with degenerative, vascular and mixed dementia also can benefit of pharmacological treatment such as cholinesterase inhibitors. Patients recruited in the study: patients admitted to the hospital between 1st of July 2002-31st of June 2003, the total of 448 patients of whom 55 were patients with MCI included in the study. The objectives were to asses the prevalence of various risk factors and co-morbidities in all new patients with MCI admitted in the memory unit, during that period and to ascertain the validity of the international model for the distribution of co-morbidities and risk factors for MCI in Romania. There were inclusion and exclusion criteria for these patients. There were charts revealing the distribution of patients regarding gender and age, education, place of residence, prevalence of alcoholism, miocardial infarction, depression, relationship between prevalence of depression and the loss of memory, prevalence of osteoporosis. The study is intended to be extended to a larger group of patients.

Keywords: *MCI, alcoholism, myocardial infarction, depression, osteoporosis.*

Introduction

In Europe, approximately 3,5 millions subjects are affected by dementia and approximately 850000 subjects will develop dementia in a year. The most frequent cause of dementia – 60% – is represented by Alzheimer's disease (Lobo et al, 2000; Henderson & Jorm, 2000).

Research on the transition between aging and AD is expanding rapidly. Before people with Alzheimer's disease (AD) become demented there is a long period in which they experience mild cognitive impairment (MCI). MCI refers to an intermediate stage of cognitive impairment, which is beyond what is expected for normal aging yet of insufficient severity to constitute dementia or AD. In this study we want to determine the prevalence of co-morbidities of MCI in the patients admitted in the Department of Geriatrics and Gerontology of "Carol Davila" University of Medicine and Pharmacy, Bucharest.

Aging is accompanied by alterations of central nervous system, that's one of the most striking change of ageing process.

With advancing age, the brain losses the neuronal tissue in a progressive fashion. This process is irreversible and is obviously accompanied by intellectual decline.

In many elderly subjects, these are measurable changes that occur with ageing, including a delay in neuromuscular response period, a decline of intelligence (which is more evident in persons who originally had lower level of intelligence than in those who had higher levels), a decrease in learning ability and loss of memory, particularly for complex materials, changes in perceptive

ability, including a decline in visual and auditory acuity.

The earlier we determine these measurable decreases in the functional capacity of central nervous system, the better we can adjust to the therapeutical procedures to delay the progressive aging of the brain. That's why it is very important to make an early diagnosis of decreases of functional capacity of central nervous system.

Mild Cognitive Impairment (MCI)

The definition of MCI is itself a "work in progress". The criteria for MCI are as follows:

1) subjective memory complaint preferentially corroborated by an informant
2) abnormal memory function relative to age and education norms
3) essentially normal general cognitive function
4) largely normal activities of daily living
5) not demented.

(Petersen et al., 2002).

Mild cognitive impairment refers to the clinical state defined by:

- memory complaint by patient, family or physician
- normal activities of daily living
- normal global cognitive function
- objective memory impairment or impairment in other area of cognitive function as evidenced by scores >1.5 SD below age appropriate norms
- CDR score 0.5
- not demented patients (MMSE >24)
- age between 60 and 89 years.

(Petersen et all, 1999, 2001).

Course of Alzheimer's Disease

Between 40-65 years we speak about asymptomatic predementia AD, between 65-70 years about symptomatic predementia AD and between 70-80 years is probable/possible AD.

Table 1. Preclinical Alzheimer's disease Scale (PAS).

Criteria	**-1**	**0**	**1**	**2**
Age	<60	60-64	65-74	>75
MMSE	-	28-30	26-27	<26
Functional impairment				
-GDS	-	1	2	3
-CDR	-	0	-	0,5
-CDR SOB	-	0	0,5-1	>1,5
Cognitive tests <50th perc.	Memory	other	1 test impaired	2 tests impaired
Medial temporal lobe atrophy				
Quantitative	-	>66%	33-66%	<33%
Qualitative	-	0	1	2
APOE genotype	-	other	1 allele APOE-e4	2 allele APOE-e4

After correction for age, Visser et all (2002).

Objectives

- To asses the prevalence of various risk factors and co-morbidities in all new patients with mild cognitive impairment admitted in the memory unit.
- To ascertain the validity of the international model for the distribution of co-morbidities and risk factors for MCI in Romania.

The study

The selection of subjects was made among the patients of The Geriatrics and Geronto-Psychiatry Department "Carol Davila", University of Medicine and Farmacy. This is the first study done in Romania on patients with Mild Cognitive Impairment (MCI) using internationally recognized diag- nostic criteria and follow-up methods.

„Carol Davila" University is the only east-European partner in the DESCRIPA project, part of European Alzheimer's Disease Consortium (EADC).

Selection of subjects

Inclusion criteria:

- All new patients referred for the evaluation of cognitive impairment who are older that 50 years and who are not demented.

Exclusion criteria:

- Subjects who meet DSM-IV criteria of dementia at baseline
- Medical or psychiatric condition causing cognitive impairment.

Data collected during clinical visit

- Demographics:
  - age, sex, education, initials
  - referral pattern, informant presence.
- Data regarding cognitive complaints:
  - year onset, course, main complaints, family history
- Data regarding non-cognitive complaints.
- Data from medical history:
  - cardiovascular, endocrine, psychiatric disorders.

Subjects evaluation criteria

- Short cognitive tests:
  - MMSE (standardized)
  - Clock test.
- Clinical rating scales:
  - CDR, CIRS.
- Neuropshycological assessment:
  - Memory: Rey AVLT
  - Language: verbal fluency
  - Executive functions: TMT B
  - Attention: TMT A

- Abstract reasoning/IQ: Raven/Token
- Visuoconstruction: Babcock/Clock
- Scoring: Raw scoring, Z-scores, Domain score.

Patients recruited in the study: patients admitted to the hospital between 1st of July 2002-31st of June 2003, the total of 448 patients of whom 55 were patients with MCI included in the study.

Data analysis

The statistical methods used were:
- two tails χ^2 test for 2 X 2 and 2 X k contingency tables
- F test for equality of two variances
- two sample t test for independent samples with unequal variances (for normally distributed variables)
- two sample test for binomial proportion.

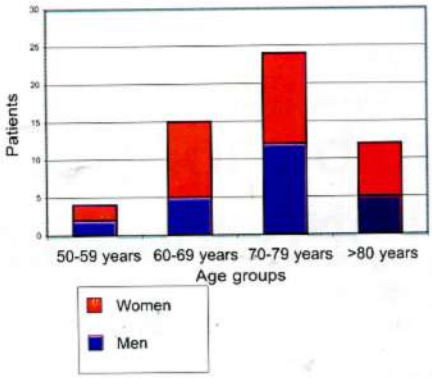
A p-value less than 0.05 was considered significant.

Discussion

Table 2 shows the relationship existing between depression and memory loss: depression was present at 26.67% of the subjects without memory loss and at 50% of the individuals with memory loss.

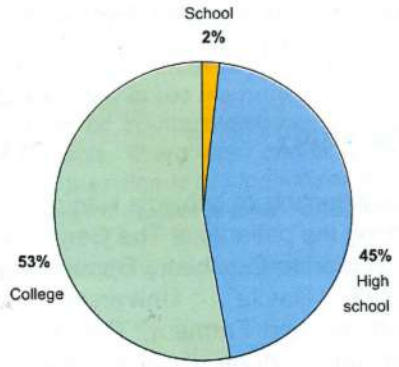
The influence of MMSE score on doctors' decision to treat depression was analyzed, but no correlations nor statistically significant differences were found (Table 3).

Figure 1. Distribution of patients by gender and age.



Regarding the risk factors for MCI, according to gender and age and respecting the worldwide data, in Romania women are more affected than men in the age groups 60-69 years, 70-79 years and above 80 years (figure 1). In the romanian MCI study, 53% of the patients attended college, 45% high school, and their place of residence was mainly urban (figures 2 and 3).

Figure 2. Distribution of patients according to their education.



As for the risk factors of MCI in Romania, the alcoholism is present among 38% of males and 6% of the females (figure 4).

In Romania, the cardiovascular mortality rate is 16%, and the prevalence of the miocardial infarction among the study population was 20.83% in men and 3.23% in women (figure 5).

Depression is a major risk factor for MCI. The romanian study shows a prevalence of depression of 54,55% in men compared to 45.45% in women from the same age group (figure 6). Among the study population, 35.48% of women were suffering from osteoporosis, while no men had this degenerative condition (figure 7).

Figure 3. Distribution of patients according to their place of residence.

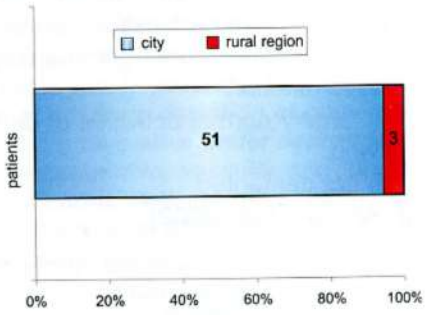


Figure 4. Alcoholism prevalence.

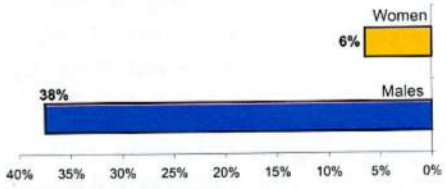


Table 2. The relationship between depression and memory loss.

	Patients with memory loss	Patients without memory loss
Prevalence of depression	26.67%	56%

Table 3. The influence of MMSE score on doctors' decision to treat depression.

	Treated depressive patients	Non-treated depressive patients
Mean MMSE	25	24.37

Figure 5. Prevalence of miocardial infarction.

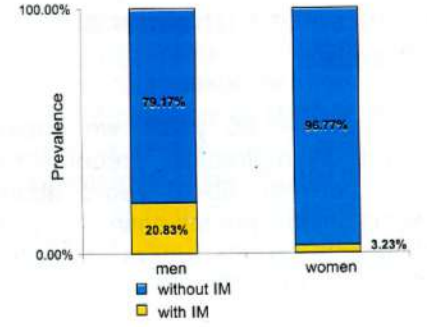


Figure 6. Prevalence of depression.

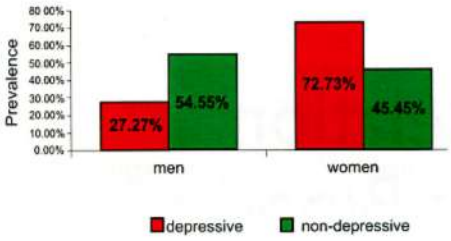
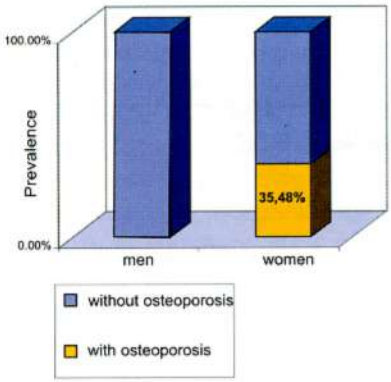


Figure 7. Prevalence of osteoporosis.



Future development

All the patients included in our study will be selected for the DESCRIPA study. (Development of Screening Guidelines and Diagnostic Criteria for Pre-dementia Alzheimer's Disease).

The subjects will be monitored in the future according to the new criteria and guidelines of follow-up from DESCRIPA study.

Conclusion

The study actively screened for MCI patients admitted in The Geriatrics and Geronto-Psychiatry Department "Carol Davila" University of Medicine and Farmacy.

Using international criteria for diagnostic, this study promoted correct evaluation and treatment of the patients, and is the first descriptive study in East-European countries on MCI. Within the study group, prevalence of co-morbidities such as cardiovascular, psychiatric or metabolic diseases, was evaluated and a model of distribution, adjusted for Romania was made.

The study showed that Geriatrics and Geronto-Psychiatry Department within the Bucharest's "Carol Davila" University of Medicine and Farmacy is able to initiate and manage a larger group study.

References

1. Petersen R.C, Doody R., Kurz A., Mohs R.C., Morris J.C., Rabins P.V., Ritchie K., Rossor M., Thal L., Winblad B. Current Concepts in Mild Cognitive Impairment, *Archives of Neurology* 2001; 58: 1985-1992.
2. Petersen R.C, Stevens J.C., Ganguli M., Tangalos E.G., Cummings J.L., DeKosky S.T. Practice parameter: Early detection of dementia: Mild cognitive impairment (an evidence-based review), *Neurology*, 2001.
3. Petersen R.C., Mild Cognitive Impairment: Transition from Aging to Alzheimer's Disease In *Neurologic Clinics*, edited by K Iqbal. West Sussex, England, J Wiley & Sons, 2001.
4. Petersen R.C. (Editor), *Mild Cognitive Impairment: Aging to Alzheimer's Disease*, New York, Oxford University Press, 2003.
5. Visser, P.J., *Predictors of Alzheimer type dementia in subjects with mild cognitive impairments*, Maastricht, 2000.
6. Kivipelto M, Helkala E-L, Hänninen T, Laakso MP, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A., Midlife vascular risk factors and late-life mild cognitive impairment. A population-based study; *Neurology*, 2001.
7. Bennett DA, Wilson RS, Schneider JA, Evans DA, Beckett LA, Aggarwal NT, Barnes LL, Fox JH, Bach J., Natural history of mild cognitive impairment in older persons, *Neurology*, 2002.
8. Palmer K., Jelic V., Winblad B., Mild Cognitive Impairment, *Acta Neurologica Scandinavica*, 2003.

Dental Amalgam Fillings in Relation to Mercury Concentration in Blood and Cognitive Function in an Elderly Urban Population

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Abstract

Background: ***Concern has been expressed regarding potential health risks associated with using mercury-containing dental fillings.***
Aim: ***The aim of this study was two-fold, (1) to evaluate the possible relationship between numbers of dental amalgam fillings and the mercury concentration in whole blood (B-[Hg]) and (2) to assess on a cross-sectional basis, the relationship between cognitive function in old age (defined by MMSE-score) and both numbers of dental amalgam fillings and B-[Hg].***
Subjects: ***Persons aged 81+ years living in a section of Stockholm (n=84), were recruited from the second follow-up of the Kungsholmen Project, a prospective cohort study on ageing and dementia. Information on numbers of dental amalgam fillings, B-[Hg] and MMSE-score was collected.***
Methods: ***The Spearman Rank correlation coefficient was used to study the relationships between numbers of dental amalgam fillings, B-[Hg] and MMSE-score. Further comparisons between groups were carried out using Student's t- and χ^2 tests.***
Results: ***A significant correlation between numbers of dental amalgam fillings and B-[Hg] was found in women but not in men. No significant correlation was demonstrated between MMSE score and either numbers of dental amalgam fillings or B-[Hg].***
Conversion factor: ***1nmol Hg/L = 0.2 µg Hg/L***
Keywords: *mercury, dental amalgam, cognition, elderly, Public Health.*

Introduction

Little is known about health effects of toxic metal exposure in aged humans. Mercury (Hg) has well documented toxic effects on the mammalian nervous and renal systems. Several sources can contribute to the Hg burden of individuals, including food, drinking water and air. Exposure from these sources can vary considerably, but it is estimated to be approximately 10-100 nmol Me-Hg/ day, 5 nmol Hg^{2+}/day and 1 nmol Hg^0/day (dental amalgam excluded) in a non-occupationally exposed population[1]. Hg^0 vapour, released from dental amalgam fillings, is another possible route of exposure[2] and is, with the exception of certain occupational exposures, considered to be the main source of human exposure to Hg^0.

The amount of Hg emitted by amalgams is in dispute, but a daily intake of 10-100 nmol Hg^0 vapour is generally expected for the average individual[3]. The main route for Hg^0 absorption is via the lungs. In humans 75-85% of the inhaled dose is absorbed[4]. Ingested Hg^0 is poorly absorbed in the gastrointestinal tract[5]. The Hg^0 that reaches the bloodstream is rapidly oxidized to Hg^{2+} in erythrocytes[6]. In that, Hg^{2+} ions do not readily cross membrane barriers, their distribution is limited. However, this oxidative pathway is saturable, and the Hg^0 that reach-

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es and penetrates other cell types, such as brain cells, is also eventually oxidized, resulting in an intracellular accumulation of Hg^{2+}. As Hg^0 is oxidized to Hg^{2+}, faeces and urine become the dominating excretion routes.

The organic form, Me-Hg, has a somewhat different kinetic profile than Hg^0. Me-Hg is effectively absorbed and readily distributed throughout the body. It easily penetrates the blood-brain and placental barriers where it is demethylated and accumulates as Hg^{2+} [7]. These features, along with its ability to bioaccumulate in wildlife, such as fish, have focused the concern for human exposure (excluding occupational exposure to inorganic-Hg) on Me-Hg. Approximately 80% of the Hg accumulated in the body is eventually excreted as Hg^{2+}. Depending on previous levels and type of Hg exposure as well as individual characteristics, the half-life of Hg in blood varies in the range of approximately 50-120 days[8,9], with an estimated mean of 65 days[10]. Hg-containing amalgam has been used as a tooth filling restoration since the early nineteenth century and is still the most common restorative worldwide. Because of the well-known toxic effects of Hg, concern has been expressed about potential health risks associated with using Hg-containing dental fillings. Amalgam has also been argued to cause a variety of adverse health effects[11], and there is an ongoing controversy about whether people experience adverse effects from the levels of mercury released from dental amalgam fillings[12-14] or not. Despite a number of previous investigations, no studies have demonstrated a clear relationship between amalgam fillings and adverse health effects[11,15-18].

Some research indicates that inhaled Hg^0 vapour, emitted from dental amalgams, contributes to the total Hg body burden, as the amount and surface area of fillings correlate with Hg levels in some body fluids of non-occupationally exposed individuals [2,11,19-21].

In recent years, three large epidemiological studies on neurotoxic effects after chronic low-dose prenatal methyl-Hg exposure have been conducted. Two of the studies[22-26] found associations between maternal consumption of fish and signs of neurotoxicity in the intrauterine exposed child, in contrast to studies reported elsewhere[27, 28].

Given the fact that doses of Hg, previously thought to be safe, might contribute to damage in the nervous system, it is plausible that a toxic injury might not reveal itself until late in life. Hg levels in brains from persons diagnosed with Alzheimer's disease (AD) have been reported to be increased[29,30] but others have not been able to replicate their findings[31]. Basun et al.[32] and Hock et al.[33] found increased levels of Hg in the blood of persons suffering from probable AD. The latter also reported that the increased blood Hg levels were unrelated to the dental status of the AD cases.

Reports on number of dental amalgam fillings and blood Hg levels in elderly are scarce in the literature. The primary aim of this paper is to evaluate the possible relationship between number of dental amalgam fillings and mercury level in whole blood (B-[Hg]), in a sample of elderly, urban-dwelling, dentate individuals participating in the Kungsholmen Project, an ongoing prospective cohort-study on ageing and dementia[34,35]. On a secondary, exploratory basis, we also assessed the relationship between each of these measures (number of dental amalgam fillings and B-[Hg]) and cognitive function.

Material and methods

Study subjects

In the present study, 84 (46 females [F], 38 males [M]) subjects were assessed for dental status and total blood-mercury concentration (B-[Hg]). The study participants represented a sub-sample of individuals participating in the Kungsholmen Project. The Kungsholmen Project started in 1987. The second follow-up occurred in 1994-1996. During this phase blood was collected for metal analysis and the oral health component Kungsholmen Elders Oral Health Study (KEOHS) was added to the ongoing project[36]. The KEOHS was restricted to generally healthy, community dwelling individuals who were then current participants in the Kungsholmen Project. Generally healthy persons were defined as individuals whose physical, medical, and mental status allowed them to travel to and participate in a comprehensive oral health examination conducted within the Kungsholmen community. Persons who lived in a nursing home, were homebound, or whose frailty precluded their travelling to the dental examination site were excluded. All identified, potentially eligible subjects were invited to join the KEOHS, first via letter and then by telephone. Reimbursement of travel expenses to and from the examination site was offered to all potential subjects. Persons who agreed to participate received an appointment that was confirmed by mail.

Out of 296 potentially eligible subjects, 159 were included in the KEOHS. Reasons for non-participation included refusal (n=71), illness (n=44), death (n=6), inability to contact the identified individual (n=5), failure to keep appointments (n=4), and other miscellaneous reasons (n=7). The most commonly expressed reason for refusal was that the potential subject was tired from previous project evaluations. A baseline examination was performed on a rolling basis after the subjects completed the parent's study second follow up. Among the 159 subjects, 129 had at least one tooth. In 84 of these 129 subjects the B-[Hg]) was known from a parallel study on toxic metals in blood in the Kungsholmen project.

There was an informed consent and approval by the local Ethical

Committee Karolinska Institutet (KI): 94:122 regarding the second follow-up in the Kungsholmen project. The consent form was approved by the applicable Institutional Review Boards.

Mercury assay

Blood-samples

Following a strict protocol[37-39], 10 ml blood was collected from each subject for metal analysis during the second follow up of the Kungsholmen Project. Each blood sample was evenly split into three acid-washed Sodium-Heparin containing polyeten tubes, frozen at –20°C for one week, and then transferred to –80°C and stored until further analyses.

Mercury analysis

During two time periods, November 1997 and July 1998, Analytica AB (present name SGAB) analysed 84 frozen blood samples, for total Hg content, from 84 subjects who also had received a KEOHS dental examination. SGAB is accredited by the Board for accrediting and technical control (SWEDAC) in accordance with Swedish law, and in compliment with the demands in SS-EN 45001 (1989), SS-EN 45002 (1989) and I80/IEC Guide 25(1990:E).

The blood samples were kept frozen at all times, described in detail elsewhere[40] until analysis. Cold vapour atomic absorption spectrometry (Milton Roy AAS-CV unit) was used to determine the B-[Hg] concentrations. After treatment with $SnCl_2$, a half millilitre of blood was analysed in duplicate. Seronorm Trace Element was used as a matrix matched quality control. The Hg-concentration of the calibration standard was 500 nmol/L, and standard was added to every fifth sample. The accredited lower limit was 10 nmol/L for both analyses (1997, 1998). In 1997, the coefficient of variance (CV) was 8% at 40 nmol/L and 17% at 15 nmol/L, and in 1998 the CV was 10% at 40 nmol/L. In 1997 the quantification limit (LOQ = 10 x SD-blank) was 8.2 nmol/L, and the detection limit (LOD = 3 x SC-blank) was 2.5 nmol/L.

Cognitive assessment, dementia diagnosis

The subjects were screened with a brief cognitive test, Mini-Mental State Examination (MMSE)[41]. The cut-off point 23/24 is usually used to discriminate between individuals suspected (MMSE ≤ 23) and non suspected (MMSE ≥ 24) demented. The dementia diagnosis was based on DSM-III-R criteria (American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, revised 3rd edn (DSM-III-R, 1987) with some modifications[34].

Dental status examination

The KEOHS baseline included both an interview and oral examination administered at either the Stockholm Long-term Care Facility or the Stockholm Gerontology Research Centre. The interview used a structured questionnaire to obtain information on subject demographics as well as dietary, lifestyle, and dental habits. The oral examination collected information on a wide array of clinical parameters including caries and periodontal status, occlusion and function, dental prosthesis status, and taste detection and recognition thresholds.

The caries examination, which identified decayed and filled tooth surfaces as well as missing teeth, was conducted by one of three standardised examiners using previously defined visual, tactile criteria for defining coronal and root caries[42]. In this study the amount of dental amalgam was evaluated by summing the total number of coronal (mesial, occusal, distal, facial, lingual) and root surfaces (mesial, distal, facial, lingual) that were restored with amalgam. Crowns with an underlying amalgam build-up and tooth surfaces restored with materials other than amalgam (e.g., gold, composite resin) were not included in the calculation of numbers of amalgam fillings. Third molars were included.

Portable dental equipment was used, and radiographs were not obtained. Examiners were standardised before and during the data collection period. Given the age of KEOHS study subjects and the length of the examination, repeat examinations to establish examiner reliability was not conducted.

Data analysis

Comparisons between groups were done with Student's t-test (unpaired, two tailed) or χ^2 test.

The Spearman Rank correlation coefficient was used to study the relationship between numbers of dental amalgam fillings and the variables B-[Hg] and MMSE score. Subjects demonstrating a B-[Hg] exceeding 28 nmol/L or below 5 nmol/L (RDL = 6 x SD of standard) were defined as outliers. Outliers were not included in the statistical analysis. Statistical significance was set at $P < 0.05$.

Results

A total of 84 subjects, 46 F and 38 M, all with ≥ 1 tooth, were assessed for number of dental amalgam fillings and B-[Hg]. Out of 84 subjects, 80 demonstrated B-[Hg] ≤ 28 nmol/L; the remaining four subjects, all male, showed extreme B-[Hg] (outliers) compared to the rest of the population (42, 55, 60 and 80 nmol/L). These outliers were excluded from the statistical analysis along with two female subjects whose B-[Hg] was < 5 nmol/L, corresponding to approximately 6 x SD of the standard (RDL). Except for B-[Hg], the exclusion of outliers (n = 6) did not change mean values of the studied variables.

Of the 84 subjects, five females were diagnosed as having AD, one male as having mixed dementia. All six subjects diagnosed with dementia were non-smokers. The B-[Hg] in these subjects ranged between

7–17 nmol/L with no relationship between lower MMSE score and higher B-[Hg]. One of the outliers (female) was diagnosed as probable AD.

Mean values (n = 78) for the variables of interest are shown in table 1.

There were no statistically significant differences between the male and female group in any of the studied variables: age (P = 0.34), B-[Hg] (P = 0.80), numbers of dental amalgam fillings (P = 0.85), MMSE score (P = 0.42), number of occlusal fillings (P = 0.47) and remaining teeth (P = 0.15), or smoking habits (χ^2 = 2.02, df. = 2, P = 5.99). Mean values of B-[Hg] and numbers of amalgam filled surfaces in each MMSE-score group are shown in figure 1.

A moderate statistically significant correlation between numbers of dental amalgam fillings and B-[Hg] was found among the females (r = 0.42, P = 0.005), but not among the males (r = 0.02, P = 0.911) (figure 2).

This gender difference remained when relating number of occlusal fillings to B-[Hg], females (r = 0.41, P = 0.006), males (r = 0.10, P = 0.57). No significant correlation was demonstrated between MMSE score and numbers of amalgam fillings or B-[Hg] (table 2). There was no obvious increase or decrease in number of teeth or amalgam fillings with increasing age.

Figure 1. Mean no. of amalgam filled surfaces and mean B-[Hg] in each MMSE-score group (n=78).

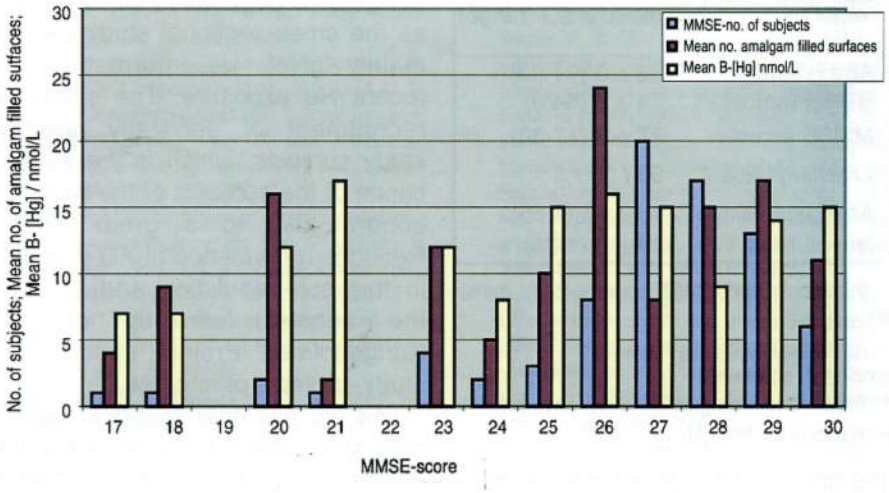
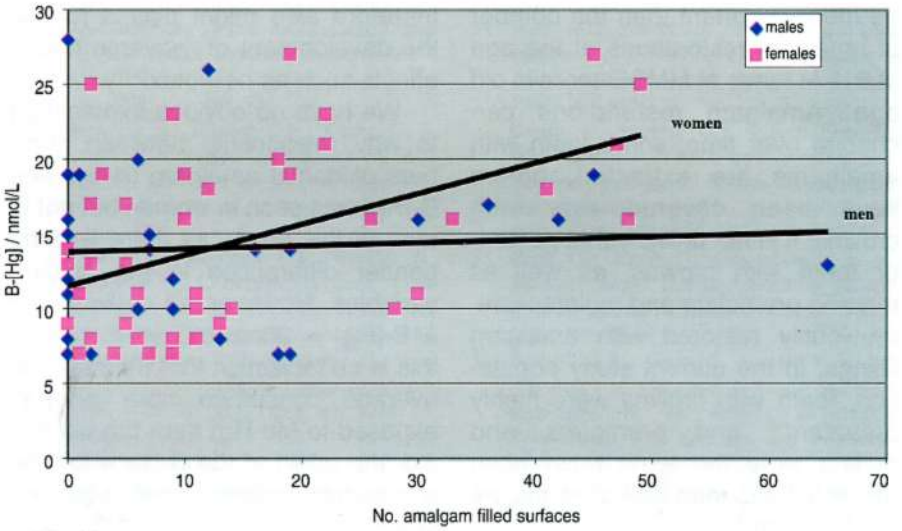


Figure 2. Relationship between numbers of amalgam filled surfaces and B-[Hg] in males (n=34) and females (n=44).



Discussion

In this study, women (n = 44) demonstrated a moderate correlation between numbers of dental amalgam fillings and B-[Hg], whilst the men (n = 34) did not. No relationship was found between MMSE-scores and either number of dental amalgam fillings or B-[Hg]. Human exposure to mercury occurs mainly as Me-Hg through eating contaminated seafood. Reported reference values for total B-[Hg] is 5-40 nmol/L[10], 10 nmol/L[43] and when considering fish consumption, 10-220 nmol/L[44].

Exposure to high levels of Hg has been associated with serious neurological and developmental effects in humans. Depending on the dose, effects can range from subtle losses of sensory or cognitive ability to death. An understanding of the potential health risks related to amalgam exposure depends upon our knowledge of the toxicology of inhaled mercury vapour and the quantities released and inhaled from amalgam restorations. Genetic and environmental conditions can influence Hg-kinetics and neurobehavioral function making risk assessment of Hg difficult, as in this study (mean B-[Hg] =14 nmol/L), especially in the lower range of the dose response curve.

There are some limitations in this study that need to be emphasised. Counting the number of amalgam filled surfaces is an indirect and blunt method of estimating exposure to Hg^0 vapour, released from dental amalgam fillings. A number of factors could have affected B-[Hg] levels in the study population including genetic differences, dietary habits, recent dental treatment, oral health status, type and location of

Table 1. Demographic statistics of total population (n = 78; arithmetic mean ± SD [range]).

Variable	mean ± SD (range)
Age (years)	86 ± 3 (81-93)
B-[Hg] (nmol/L)	14 ± 6 (6-28)
MMSE score*	27 ± 3 (17-30)
Smoking habits**	59 / 14 / 5
Amalgam fillings***	13 ± 15 (1-65)
No. of teeth	17 ± 7 (1-28)

* 9 subjects had MMSE-score < 24 (3 male; 6 females)

** No. of non smokers / stopped smoking / smokers

*** Total no. of amalgam filled surfaces (21,4% were occlusal fillings).

the amalgam fillings as well as medical status. Further estimating B-[Hg] primarily reflects recent Hg exposure (all types).

Amalgams placed years ago may be more important than the number of amalgam restorations at the age of 81+ in terms of MMSE score in old age. Amalgam restorations can change over time; some teeth with amalgams are extracted, others have been covered over with crowns. It is not unlikely that posterior teeth with crowns, as well as missing premolars and molars were previously restored with amalgam fillings. In the current study population, teeth with crowns were highly prevalent[45] and premolars and molars were the teeth most often missing. Less than half of all molars were retained.

The fact that no relationship was found between MMSE-scores and either numbers of dental amalgam fillings or B-[Hg] would be expected as the cross-sectional study design mainly gives us information on recent Hg exposure. The selective recruitment of generally healthy study subjects, which is the significance of the subjects of the KEOHS cohort, also to a great extent excludes (prevalence of AD was 5% in this sub-population and 27% in the second follow-up of the Kungsholmen Project cohort) the study-subjects of interest.

As we however found a significant relationship between numbers of dental amalgam fillings and B-[Hg] in women the possibility exists that a chronic low-dose exposure from Hg-amalgam might contribute to the total lifetime body-burden of Hg and therefore also might play a role in the development of adverse health effects such as neurotoxicity.

We have no obvious explanation to why relationship between numbers of dental amalgam fillings and B-[Hg] was seen in women but not in men in this study, as there was no gender differences in the studied variables. However, all outliers with a B-[Hg] > 28nmol/L were men. If this is an indication that the men, on average, consumed more fish (i.e. exposed to Me-Hg) than the women did, the origin of the B-Hg would be somewhat different between the sexes. Hg-amalgam would then to a greater extent explain the B-[Hg] in the women than in the men. Differences in kinetics might also be a plausible explanation to the observed gender difference in the relation B-[Hg] vs. numbers of dental amalgam fillings.

The results must however be interpreted with great caution as the study population was both small and selectively recruited.

Conclusions

Our results indicate that numbers of dental amalgam fillings, in part, might explain the B-[Hg] level. Therefore, the possibility that a chronic low-dose exposure, from Hg-amalgam, contribute to the total lifetime body-burden of Hg cannot be ruled out.

Studying and making risk assessment of Hg exposure is difficult especially in the lower range of the dose response curve. Considering reports of elevated B-[Hg] levels in AD patients and the well documented neurotoxic effects of Hg exposure, further research is needed to identify health consequences of long-term exposure to low levels of Hg, as well as potential risk groups.

No studies to date have proven any connection between Hg-amalgam fillings and adverse health effects. This indicates a need also to introduce new biological markers, both for exposure and effects of Hg,

Table 2. Correlation coefficients (Spearman Rank type) between numbers of dental amalgam fillings, no. occlusal fillings and the variables B-[Hg] and MMSE-score.

Subjects	Amalgam fillings* vs. B-[Hg].	No. occlusal fillings* vs. B-[Hg].	Amalgam fillings* vs. MMSE score.	No. occlusal fillings vs. MMSE score.	B-[Hg] vs. MMSE score.
Females (n=44)	0.42 (P=0,005)	0.41 (P=0.006)			0.06 (P=0.691)
Males (n=34)	0.02 (P=0.911)	0.10 (P=0.573)			0.24 (P=0.179)
Tot. (n = 78)	0.24 (P=0.032)	0.27 (P=0.016)	0.02 (P=0.869)	0.04 (P=0.74)	0.12 (P=0.290)

* Total no. of amalgam filled surfaces.

to bring in more aspects in the risk assessment procedure.To better understand the interaction between ageing and exposure to chemicals, concerning the development of adverse health effects, we need to increase our knowledge regarding kinetics and biological parameters in elderly people[46]. Thus this study gives a contribution to this knowledge and has its merits in reporting on B-[Hg] levels and numbers of dental amalgam fillings in an well defined elderly urban population.

Acknowledgements

Professor Laura Fratiglioni and Professor Douglas E. Morse are gratefully acknowledged for their support of the project. We thank all the members in the Kungsholmen Study Group for their collaboration and for financial support from Karolinska Institutet and Alzheimer foundation, Sweden.

References

1. WHO, Guidelines for drinking water quality, Mercury 2nd ed. Vol.2 *Health criteria and other supporting information*. World Health Organisation, Geneva 1996.
2. Aronsson A.M., Lind B., Nylander N.,Nordberg M., Dental amalgam and mercury. *BioMetals* 1989; 2: 25-30.
3. Begerow J., Zander D., Freier I., Dunemann L., Long-term mercury excretion in urine after removal of amalgam fillings. *Int Arch Occup Environ Health* 1994; 66(3): 209-212.
4. Hursh J.B., Partition coefficients of mercury (^{208}Hg) vapour between air and biological fluids. *J Appl Toxicol* 1985; 5(5): 327-332.
5. Bornman G., Henke G., Alfes H. et al., Concerning the enteral absorption of metallic mercury. *Arch Toxicol* 1970; 26: 203-209.
6. Halbach S., Clarkson T.W., Enzymatic oxidation of mercury vapor by erythrocytes. *Biochem Biophys Acta* 1978; 522-531.
7. NRC-National Research Council, *Toxicological Effects of Methylmercury*. National Academy Press Washington, DC. USA 2000.
8. Birke G., Johnels A.G., Plantin L-O., Sjostrand, Skerfving S., Westermark T., Studies on humans exposed to methyl mercury through fish consumption. *Arch Environ Health* 1972; 25: 77-91.
9. Scherlock J., Hislop J., Newton D., Topping G., Whittle K., Elevation of mercury in human blood from controlled chronic ingestion of methylmercury in fish. *Hum Toxicol* 1984; 3: 117-131.
10. WHO/IPCS, Methylmercury. *Environ Health Criteria 101*, IPCS, World Health Organisation, Geneva 1990.
11. WHO/IPCS, Inorganic Mercury. *Environ Health Criteria 118*, IPCS, World Health Organisation, Geneva 1991.
12. Echeverria D., Aposhian H.V., Woods J.S., Heyer N.J., Aposhian M.M., Bittner A.C. Jr. et al., Neurobehavioral effects from exposure to dental amalgam Hg (0): new distinctions between recent exposure and Hg body burden. *FASEB J* 1998; 129: 971-80.
13. Eley B.M., The future of dental amalgam: a review of the literature. Part 5: mercury in the urine, blood and body organs from amalgam fillings. *Br Dent J* 1997;182: 413-417
14. Jones D.W., Exposure and absorption and crucial question of limits for mercury. *J Can Dent Assoc* 1999; 65: 42-46.
15. U.S. Public Health Service, Dep of Health and Human Services, *Dental amalgam: A Scientific Review and Recommended Public Health Service Strategy for Research, Education, and Regulation*. Washington: US DHHS 1993.
16. SoS-Socialstyrelsen (The National Board of Health and Welfare) redovisar 1994:21 (in Swedish, summary in English) ISBN 91-38-11407-0.
17. Forskningsrådsnämnden 1998 (in Swedish).
18. ADA- American Dental Association, Dental Amalgam: update on safety concerns. ADA Council of Scientific Affairs. *J Am Dental Assoc* 1998; 129: 494-503.
19. Langworth S., Elinder C.G., Gothe C. J., Vesterberg, Biological Monitoring of Environmental and Occupational Exposure to Mercury. *Arch Occup Environ Health* 1991; 63: 161-167.
20. Olstad M.L., Hooland R.I., Wandel N., Hengsten P A., Correlation between Amalgam Restorations and Mercury Concentrations in Urine. *J Dent Res* 1987; 66: 1179-1182.
21. Snapp K.R., Boyer D.B., Peterson L.C., Svare C.W., The Contribution of Dental Amalgams to Mercury in Blood. *J. Dent Res* 1989; 68: 780-785.
22. Kjellström T., Kennedy P., Wallis S., Mantell C., Physical and mental development of children with prenatal exposure to mercury from fish. National Swedish Environmental Protection Board report 3080, Stockholm, Sweden 1986.
23. Kjellström T., Kennedy P., Wallis S. et al., Physical and Mental Development of children with Prenatal Exposure to Mercury from Fish. Stage II: Interviews and psychological tests at age 6. *National Swedish Environmental Protection Board report* 3080, Stockholm, Sweden 1989.
24. Grandjean P., Weihe P., White R.F. et al., Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; 19(6): 417- 428.
25. Grandjean P., Weihe P., White R.F., et al., Prenatal exposure to methylmercury in the Faroe Islands and neurobehavioral performans at age seven years. Response to workgroup questions for presentation on 18-20 Nov. 1998. *In Scientific Issues Relevant to Assessment of Health Effects from Exposure to Methylmercury*. Appendix II-B, Faraoe Islands Studies. National Institute for Environmental Health Sciences 1998.
Available:http//ntp-server.niehs.nih.gov/main_pages/PUBS/MethMercWshpRpt.html.
26. Grandjean P., Budtz-Jorgensen E., White R.F., Weihe P., Debes F., Keiding N., Methylmercury exposure biomarkers as indicators of neurotoxicity in children age 7 years. *Am J Epidemiol* 1999; 150(3): 301-305.
27. Davidson P.W., Myers G.J., Cox C., et al., Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion: outcomes at 19 and 29 months. *Neurotoxicol* 1995; 16(4): 677-688.
28. Davidson P.W., Myers G.J., Cox C. et al., Effects of prenatal and postnatal methylmercury exposure from fish comsumption on neurodevelopment: Outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA* 1998; 280(8): 701-707.
29. Ehmann W.D., Markesbery W.R., Alauddin M., Hossain T.I., Brubaker E.H., Brain trace elements in Alzheimer's disease. *Neurotoxicol* 1986; 7(1): 195-206.
30. Thompson C.M., Markesberry W.R., Ehmann W.D., Mao Y.X., Vance D.E., Regional brain trace-elements studies in Alzheimer's disease. *Neurotoxicol* 1988; 9(1):1-7
31 Ward N.I., Mason J.A., Neutron activation analisis for identifying elemental status in Alzheimer's disease. *J Radiol Nucl Chem* 1987; 113: 515-526.
32. Basun H, Forsell G.L., Wetterberg L., Winblad B. Metals and trace elements in plasma and cerebrospinal fluid in normal ageing and Alzheimer's disease. *J Neural Transm* 1991; 4: 231-258.
33. Hock C., Drasch G., Golombowski et al., Increased blood mercury levels in patients with Alzheimer's disease *J Neural Transm* 1998; 105: 59-68.
34. Fratiglioni L., Grut M., Forsell Y., et al., Prevalence of Alzheimer's disease and other dementias in an elderly population: Relationship with age, sex, and education. *Neurol* 1991; 41: 1886-1892.
35. Fratiglioni L., Viitanen M., Bäckman L., Sandman PO., Winblad B., Occurrence of Dementia in Advanced Age: The Study Design of the Kungsholmen Project. *Neuroepidemiol* 1992; 11(suppl 1): 29-30.
36. Morse D.E., Holm-Pedersen P., Holm-Pedersen J., et al., Dental caries in persons over the age of 80 living in Kungsholmen, Sweden: Findings from the KEOHS Project. *Community Dental Health* 2002a; 19: 261-267.
37. Basun H., Lind B., Nordberg M., Nordström M., Sparring-Bjorksten K., Winblad B., Cadmium in blood in Alzheimer's disease and non-demented subjects. *BioMetals* 1994; 7: 130-134.
38. Nordberg M., Winblad B., Fratiglioni L., Basun H., Lead Concentrations in Elderly Urban People Related to Blood Pressure and Mental Performance: Results From a Population Based Study. *Am J of Industrial Medicine* 2000a; 38: 290-294.
39. Nordberg M., Winblad B., Basun H., Cadmium concentration in Blood in An

Elderly Urban Population. *BioMetals* 2000b; 13: 311-317.

40. Johansson N., Basun H., Winblad B., Nordberg M., Relationship between mercury concentration in blood, cognitive performance, and blood pressure, in an elderly population. *BioMetals* 2002; 15: 189–195.
41. Folstein M.F., Folstein S.E., Mc Hugh P.R., 'Mini-Mental State': a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189.
42. U.S. Dep of Health and Human Services (USDHHS), PHS, National Institutes of Health (1987) Oral Health of United States Adults. *The national survey of oral health in U.S. employed adults and seniors: 1985-1986, National Findings.* Bethesda, MD, NIH Publication No. 87-2868.
43. Nordberg G.F., Brune D., Gerhardsson L. et al., The ICOH and IUPAC international program for establishing references values of metals. *Sci Total Environ* 1992; 120(1-2): 17-21.
44. Brune D., Nordberg Gr., Vesterberg et al., A review of normal concentration of mercury in human blood. *Sci Total Environ* 1991; 100: 235-282.
45. Morse D.E., Holm-Pedersen P., Holm-Pedersen J., et al., Prosthetic crowns and other clinical risk indicators of caries among old-old Swedish adults: Findings from the KEOHS Project. *Gerodontology*, 2002b; 19(2): 73-79.
46. WHO/IPCS, Principles for Evaluating Chemical Effects on the Aged Population. *Environmental Health Criteria* 144, IPCS, World Health Organisation, Geneva 1993.

Can (Resistance) Exercise Training Improve Cognitive and Physical Function in an Elderly Patient with Dementia?

A CASE REPORT

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Background

Although a fact too often overlooked, cognitive function and physical function are *both* important and conjoined domains of functioning in the dementia syndrome[1]. The neurodegeneration that is characteristic of dementia typically manifests in the gradual deterioration of memory, language function, other intellectual abilities, as well as normal social, occupational, and physical function[2]. Current pharmacologic treatment options (primarily AChI) to abate the cognitive decline in dementia are limited, and in any case inadequately address the intrinsic decline in physical function. Alternative therapies that might target both the cognitive and physical deterioration are thus warranted.

Recent evidence from large population-based epidemiologic studies suggests a protective effect of exercise against cognitive decline and dementia onset[3-7]. Exercise interventions have a strong potential to be beneficial for people with dementia. Even before the onset of cognitive decline, restriction in activity in community dwelling elders is recognized as a risk factor for ADL disability[8]. Reductions in strength and muscle mass also represent a major factor in loss of functional ability, particularly in patients with cognitive impairment[9]. Declines in muscle mass and strength are associated with decreased postural balance and impaired gait, glucose intolerance, loss of bone mineral density, predisposing the individual to catastrophic injuries such as fall, increasing functional limitations and metabolic disorders.

Exercise has increasingly been demonstrated as a method for maintaining and recovering function in elderly populations. Most early work in this area excluded demented individuals, but did show that exercise in different forms (resistance, aerobic) could contribute to improved physical function as well as reduce the risk of depression.[10] (Singh et al, *J of Gerontology,* 2001). More recent work with patients with dementia has shown a positive effect of exercise participation or training on physical function[11-17]. Although a recent trial also indicates the potential of exercise training combined with behavioural management to improve physical health and depression among patients with AD[18], the question of whether exercise can improve cognition as well as physical function in patients with manifest dementia remains unresolved.

This report describes the physical and cognitive benefits of a 16-week resistance exercise training program for an 85-year old woman resident in an Alzheimer's boarding home in the north-eastern United States.

Case history

Background information: Mrs. D. age = 84, 1.51 m, 67.27 kg, BMI = 29.5, Waist/Hip Ratio = 0.77, no reported weight loss in previous 3 months, MNA-SF = 12, B_{12} level = 286], had been living alone in her own home since becoming widowed in her late 60's. She had been in good health her whole life except for

having had a total abdominal hysterectomy and a cholecystectomy in the remote past, and surgeries 10 years apart for bladder neck suspension and cystocele repair. She also suffered from cataracts, and hypercholesterolemia. She took nadolol for hypertension (typical range in records was 140-150/60-80 on medication). She had no previous psychiatric history and benign levels of alcohol use. She did not smoke, having quit after her husband died. She had an active social life and good relationships with her neighbors, her sons and her grandchildren. Family history was negative for any dementing illness or psychiatric disorder, and in fact, she had a 90 year-old sister who was living independently in the community.

However, shortly after she turned 81, she began going to soup kitchens because she was convinced (wrongly) that she had no money. She frequently did not recognize her home and she often appeared to her sons to forget that her husband had died. She had incontinence of urine that was evaluated by a urologist who found no cystocele and a well-supported bladder neck. She continued to live at home with assistance from her sons.

A year later, she showed a marked decline in her ability to care for herself. She could dress herself, but tended to wear the same clothes every day. She was convinced that she had two houses and would pack clothes to go between them, when, in reality, she would become lost while driving and not recognize her house when she managed to find it again. Her family noted her to be confused, more irritable with neighbors, and she, a retired accountant, was no longer able to do simple arithmetic. She had also begun to experience falls, although a physical exam from that time notes no gait abnormalities, no tremors and no cogwheeling. An MRI obtained by the family physician showed mild generalized cortical atrophy, and periventricular and subcortical changed suggestive of small vessel occlusive disease. No laboratory abnormalities were reported in her old record. A CBC, urinalysis, TSH, B_{12} and serum chemistries had been ordered.

According to her medical record, she was diagnosed with a "subcortical dementia" and was started on rivastigmine. After 2 months on the acetylcholinesterase inhibitor, her functional status had deteriorated. Her family had been frantic with worry because she had been missing for a full day when she got lost driving. She was demonstrating confusion about real events versus events on television, thinking that the TV people were in the room with her. She was convinced that people were breaking into her house. She continued to dress and bathe herself, but her hygiene had deteriorated.

At this point, her sons decided that they could no longer keep her safe at home. She moved into her first boarding home where the rivastigmine was stopped. She began to exhibit aggression toward other residents of the facility. She was given zyprexa (dose not known), and eventually brought to the emergency room of the local hospital when her level of violence exceeded what the facility could tolerate. At this point, new placement was sought, and she moved to her current facility, where we encountered her in November, 2002.

Her admission documents to the current facility note that she wandered, was restless and resistive of ADL cares with occasional striking out at staff, and needful of frequent re-direction. She continued to have incontinence of urine. She was not demonstrating any indicators of depression. The admission physical exam notes that she appeared to lean to the right while sitting and she had slight facial droop on the side. She had no cogwheeling, no resting tremor, and she was able to ambulate without assistance. Her medications included only nadolol 120 mg po q day and as-needed 0.5 mg of ativan, which was rarely used. After 2 weeks in the facility, she was given ambien, 5 mg q HS for sleep. At this time, her diagnosis was modified to vascular dementia.

Mrs. D was randomized to the experimental group of an exercise study of non-institutionalized people with dementia (after obtaining permission from her care givers).

Exercise intervention

The resistance-exercise training (RET) program consisted of sixteen weeks of exercise using Theraband®, a brand of elastic resistive band. Each subject was encouraged to complete (up to a maximum of) three sessions per week of training and received one-on-one supervision throughout the exercise period. The exercise training was performed by facility staff under the supervision of the activities co-ordinator, who was rigorously trained to perform and teach the exercises, and to gauge the performance of the subject so that it could be recorded.

Following a brief warm-up, each subject completed 12 exercises to target the hip flexors, hip extensors, hip abductors, hip adductors, knee flexors, knee extensors, ankle dorsiflexors and ankle plantarflexors. The exercises were designed to be completed in sitting and standing positions. Each exercise was performed for 1 set of 15 repetitions. The Theraband® was color coded in the following order of increasing resistance: red, green, blue, black and silver. Each subject began the program using the red Theraband®. As a subject demonstrated the ability to complete 15 repetitions without difficulty, he/she progressed to the next Theraband® color. The degree of strength gain attainable in older subjects is dependent upon the intensity of the training stimulus. Thus, to maintain the intensity of the stimulus, the load for a particular muscle was increased to the next color of Theraband when the subject was able to perform 15 repetitions in

proper form without overexertion at a given color of Theraband. Each exercise session began with ten minutes of warm-up exercises focused on flexibility and coordination and, after the completion of the resistance training regimen, concluded with five minutes of cool-down activities. The subject was taught and supervised that each repetition should last from six to nine seconds, separated by a two second rest between repetitions. Exercise attendance records were kept to check adherence. Records also noted any progression in the level (i.e., color) of Theraband® resistance.

Participation in exercise training
Mrs. D participated in 30 out of 48 possible exercise sessions, refusing to participate on 10 other occasions. She progressed, across the 16 weeks, in the level of resistance which she was able to tolerate.

Response to exercise training

Physical: Her Timed-Up-and-Go Test time decreased 11.22 seconds to 24.16 to 12.94. Similarly, her habitual gait time over 6m decreased 3.97 seconds from 11.53 (with 20 steps) to 7.56 (14 steps) and her maximally safe gait time decreased 1.75 seconds from 9.97 (18 steps) to 8.22 (14 steps).Her sit-to-stand time (1 repetition) declined from 5.06 seconds to 2.09. Her hand-grip strength (averaged across both hands) increased from 9 kg/m to 12 kg/m. Her 360° turn time decreased from 8.78 seconds (with 13 steps) to 5.85 (10 steps).

Mood: By all measures, her mood had remained stable over the 6 month interval between assessments. Her initial geriatric depression scale score was 5, ending score 8 and the Cornell Scale for Depression in Dementia was 4 in Dec 02, 5 in June 03.

Cognition: Stable to improved on all tests except the Mattis Dementia Rating Scale-2, which showed a 2-point improvement in memory at the end of 6 months, from 10 to 12 (20 maximum), but an 8-point decline in tests of attention (37 maximum) for an over-all drop in 6 points to 95 (139 maximum possible). Her MMSE increased by 11 points, from an initial score of 18 to 29. Her clock drawing test improved by 1 point from a initial assessment of 3 to the maximum of 4.

Neuropsychologic performance: She improved on the Trails making motor-speed portion from 150 to 125 seconds. Her Hopkins Delayed Recall (0 initially and also at post-test) was stable, while her Hopkins Recognition – True Positive increased from 9 of 12 to 10 of 12. On tests of verbal fluency, her letter test improved by 14 points (total possible???). Her Stroop test scores also improved, from 43 and 37 seconds on the 90 second (max.) colour-naming and word-reading, respectively, to 35 and 26 seconds. Her Verbal Fluency scores also increased uniformly.

Overall function: Her Clinical Dementia Rating scale, which rates the patient on 6 functional domains with scores of 0 for no impairment to 3 for severe impairment, improved from a 2 initially (sum of boxes, 12), to a 1 (sum of boxes, 7) six months later.

Discussion

This presentation describes the potential benefit of sustained exercise training in an 85 year old woman with functional dementia, and untreated with antidepressant or cholinesterase inhibitor therapy. The dramatic improvements observed in cognitive and, particularly, physical function, suggest that such changes can be reasonably attributed to either the exercise-training intervention or to the change in her environment, or a combination of these factors.

Fiatarone and Evans[19], as well as other investigators in subsequent studies, have indicated the considerable benefit in physical function and vitality to moderate intensity resistance-training in even frail and previously non-active nonagenerians. Although long-term aerobic exercise training has been shown to have no effect on the psychological function of older adults[20] some recent evidence indicates that physical activity induces the expression of trophic factors in select brain regions related to higher cognitive function. It is possible that these elevated levels of trophic factors may facilitate cognitive development[21]. As part of the protocol of the study involving this patient, serum levels of BDNF and IGF-1 were also evaluated. Comparisons of levels pre- and post-exercise training indicate up-regulation of IGF-1 (0.23 ng/ml) but down-regulation of BDNF (-1.8 ng/ml). Sedentary lifestyles and nominal mental stimulation are related to low levels of BDNF and IGF-1 and to an increased incidence of AD[22]. Many of the neurological and behavioral symptoms appear to be due to neural/glial cell dysfunction or death caused by altered signal transduction processes[23]. Much evidence now indicates that neurons require multiple trophic factors to maintain normal function, yet clinical studies on this topic are lacking

Alzheimer's disease is one of the most costly conditions to afflict elderly people. The cost of providing care for people with dementia exceeds that of almost all other conditions[24-25]. The chief factor driving these costs is the provision of institutional long term care[26-27]. The advent of treatments for dementia that forestall dependence in any of a number of areas will relieve the financial pressures on families and societies by delaying the rate of progression to severe dementia that typically warrants institutionalization, with its attendant high costs, as well as lessen the care giving burden for those patients

Table 1. Pre- and Post-Intervention Results for Mrs. D.

	Pre-Intervention	Post-Intervention	Intended Direction Change	Magnitude of Change
Body Fat%	31%	33.4%		↑2.4%
GDS	7	8	↓	↑1 point
Pittsburgh Agitation	0	0		—
CDT	3	4	↑	↑ 1 point
MMSE	18	29	↑	↑ 11 points
Mattis Total	101	95	↑	↓ 6 points
Mattis Attention	35	27	↑	- 8 points
Mattis Initiat/Persev	17	17	↑	—
Mattis Construction	6	6	↑	—
Mattis Conceptualization	33	33	↑	—
Mattis Memory	10	12	↑	2 points
Hopkins Del Recal	0	0	↑	—
Hopkins True Positive	9	10	↑	1 word
Stroop 90 sec Col Naming	43	35	↓	↓ 8 sec
Stroop 90 sec Word Recog	37	26	↓	↓ 11 sec
Trails Motor Speed	—	125 sec	↓	Able to complete
Trails Vision	150 sec	42 sec	↓	↓ 108 sec
Trails Num Seq	150 sec	128 sec	↓	↓ 22 sec
Trails Let Seq	150 sec	150 sec	↓	—
Trails Switching	—	—	?	—
Verbal Fluency Letter	4	18	↑	↑ 14 points
Verbal Fluency Category	8	14	↑	↑ 6 points
Verbal Fluency Switching	2	6	↑	↑ 4 points
Left grip (kg force)	7	8	↑	↑ 1 kg force
Right grip (kg force)	11	16	↑	↑ 5 kg force
Sit-to-stand (secs)	5.06	2.09	↓	↓ 2.97 sec
6m Usual gait time (secs)	11.53	7.56	↓	↓ 3.97 sec
6m Usual gait steps	20	14	↓	↓ 6 steps
6m Fast gait time (secs)	9.97	8.22	↓	↓ 1.75 sec
6m Fast gait steps	18	14	↓	↓ 4 steps
TUG (secs)	24.16	12.94	↓	↓ 11.22 sec
360° turn (secs)	18.78	5.85	↓	↓ 2.93 sec
360° turn (steps)	13	10	↓	↓ 3 steps
Tinetti balance score	12	15	↑	↑ 3 points
Tinetti gait score	10	10	↑	—

with mild to moderate dementia who usually continue to live in the community. Exercise has well-established health-related benefits, including protection against all-cause mortality, cardiovascular illness, and the maintenance of overall health status. The accumulation of evidence that is suggestive of a protective effect of exercise against cognitive decline and dementia onset should only be heralded.

Acknowledgements

Funding for the exercise-training program was provided to VS Thomas by the Alzheimer's Association of America (**IIRG-01-3132)** and the Thera-Band: The Hygienic Corporation

References

1. Thomas VS. Excess functional disability among demented subjects?: Findings from the Canadian Study of Health & Aging. Dement Geriatr Cogn Disord 2001; 12(3):206-210.
2. APA.Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Wash,DC, 1994.
3. Yaffe K, Barnes D, Nevitt M, Lui LY, Covinsky K. A propsective study of physical activity and cognitive decline in elderly women. Arch Intern Med 2001; 161:1703-1708.

4. Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons Arch Neurol 2001; 58:498-504.
5. Fabrigoule C, Letenneur L, Dartigues JF, et al. Social and leisure activities and risk of dementia: A prospective longitudinal study. J Am Geriatr Soc 1995; 43:485-490.
6. Wang H-X, Karp A, Winblad B, Fratiglioni L. Late-life engagement in social and leisure activities decreases the risk of dementia: A longitudinal study from the Kungsholmen Project. Am J Epidemiol 2002.
7. Verghese J, Lipton RB, Katz MJ, et al. Leisure activities and the risk of dementia in the elderly. N Engl J Med 2003; 348(25):2508-2516.
8. TM, Allore H, Guo Z. Restricted activity and functional decline among community-living older persons. Arch Intern Med 2003; 163:1317-1322.
9. Dvorak RV, Poehlman ET. Appendicular skeletal muscle mass, physical activity and cognitive status in patients with Alzheimer's disease. Neurology 1998; 51:1386-1390.
10. Singh NA, Clements, KM, Singh MAF. The efficacy of exercise as a long-term antidepressant in elderly subjects: A randomized, controlled study. J of Gerontology 2001; 56A(8): M497-M504.
11. Brill PA, Drimmer AM, Morgan LA, Gordon NF. The feasibility of conducting strength and flexibility programs for elderly nursing home residents with dementia. Gerontologist 1995; 35:2:263-266.
12. Teri L, McCurry SM, Buchner DM, et al. Exercise and activity level in Alzheimer's disease: a potential treatment focus. J Rehabil Res Dev 1998; Oct;35(4):411-9.
13. Arkin SM. Elder rehab: a student-supervised exercise program for Alzheimer's patients. Gerontologist 1999; Dec;39(6):729-35.
14. Rolland Y, Rival L, Pillard F et al. Feasibility of regular physical exercise for patients with moderate to severe Alzheimer's disease. J Nutr Health Aging 2000; 4(2):109-113.
15. Thomas VS, Hageman PA. Reliability of physical performance measures in elderly day-care center clients with dementia. Int Psychogeriatr 2002; 14:1:17-24.
16. Hageman, PA, Thomas VS. Gait performance in dementia: The effects of a 6-week resistance training program in an adult day-care setting. Int J Geriatr Psychiatr 2002; 17(4): 329-334.
17. Thomas VS, Hageman PA. Can neuromuscular weakness in people with dementia be rehabilitated using resistance exercise training? Results from a preliminary intervention study. J Gerontol: Med Sci 2003; 58A:746-751
18. Teri L, Gibbons LE, McCurry SM, Logsdon RG, Buchner DM et al. Exercise plus behavioral management in patients with Alzheimer Disease. JAMA 2003; 290(15):2015-2022.
19. See Fiatarone MA, Evans WJ. The etiology and reversibility of muscle dysfunction in the aged. J Gerontol 1993; 48(Special Issue):77-83.
20. Hill RD, Storandt M, Malley M. The impact of long-term exercise training on psychological function in older adults. J Gerontol 1993; 48:12-1
21. Gomez-Pinilla F, So V, Kesslak JP. Spatial learning and physical activity contribute to the induction of fibroblast growth factor: Neural substrates for increased cognition associated with exercise. Neuroscience 1998; 85:1:53-61.
22. Gomez-Pinilla F, Ying Z, Roy RR, Molteni R, Edgerton VR. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. J Neurophysiol. 2002. Nov; 88(5):2187-95.
23. Cotter RL, Burke WJ, Thomas VS, et al. New insights into the neurodegenerative process of Alzheimer's disease: mononuclear phagocyte-associated inflammation and neurotoxicity. J Leukocyte Biology 1999; 65:4:416-427.
24. Canadian Study of Health and Aging Working Group. Canadian study of health and aging: study methods and prevalence of dementia. Can Med Assoc J 1994; 150 (6): 899-913.
25. Ostbye T, Crosse E. Net economic costs of dementia in Canada Can Med Assoc J 1994; 151(10): 1457-1464
26. Coughlin TA, Liu K. Health care costs of older persons with cognitive impairments. Gerontologist 1989;29(2) 173-182.
27. Ernst RL, Hay JW, Fenn C, Tinkleberg J, Yesavage JA. Cognitive function and the costs of Alzheimer disease. Arch Neurol 1997; 54:687-693.