Master Thesis

Title: The relationship between the 'use it or lose it' theory and the mechanisms underpinning Alzheimer's disease.

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Abstract

In light of recent research, which has demonstrated a significant energy deficiency in the brains of individuals with Alzheimer's disease (AD), it has been proposed that stimulation of the brain will slow down brain aging and thus diminish the risk for Alzheimer's disease, a principle known as the 'use it or lose it' theory. This notion, however, might seem to contradict the long-withstanding amyloid cascade hypothesis, which proposes that amyloid-β peptide plaques, found in individuals with AD, are the culprit of Alzheimer's disease. However, this review demonstrates that the energy deficiency experienced in AD due to age-related mitochondrial dysfunction can actually be linked to the amyloid cascade hypothesis in a rather intricate way. This review also discusses the cognitive and physiological benefits of physical exercise and an engaging lifestyle – two practical approaches of the 'use it or lose it' theory – and describes how key elements of the research discussed prior are involved in these benefits.

List of abbreviations

8-OHG	8-hydroxydeoxyguanosine
(α- KGDHC)	α-ketoglutarate dehydrogenase complex
Aβ	Amyloid-β protein
ABAD	Aβ-binding alcohol dehydrogenase
AβOs	Aβ oligomers
acetyl-CoA	acetyl co-enzyme A
ADDLs	Aβ-derived diffusible ligands
AD	Alzheimer's disease
ADP	adenosine diphosphate
AICD	APP intracellular domain
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APH1a	Anterior Pharynx Defective 1
apolipoprotein E	apoE
APP	amyloid precursor protein
ATP	adenosine -5'-triphosphate
BACE1	β-site APP cleaving enzyme 1
BST	basal synaptic transmission
CA1	callicebus cornus ammonis 1
CamKII	calcium/calmodulin dependent kinase II
CBV	cerebral blood volume
CMRglu	cerebral metabolic rate of glucose
CNS	central nervous system
CO_2	carbon dioxide
CSF	cerebral spinal fluid
COX	cytochrome oxidase
CR	control region

CREB	cyclic AMP response element binding protein
Cu/ZnSOD	copper/zinc superoxide dismutase
EE	environmental enrichment
ELISA	enzyme-linked immunosorbent assay
ERP	event related potential
ETC	electron transport chain
FAD	flavin adenine dinucleotide
FDG–PET	fludeoxyglucose-Positron Emission Tomography
fMRI	functional Magnetic Resonance Imaging studies
GSH	reduced glutathione
GSSG	oxidized glutathione
HCN cells	human cortical neuronal cells
$HO_2\cdot$	Hydroperoxyl
H_2O_2	hydrogen peroxide
IGF	insulin-like growth factor
LTD	long-term depression
LTP	long-term potentiation
mtDNA	mitochondrial DNA
mtRNA	mitochondrial RNA
NAD	nicotinamide adenine dinucleotide
NCT	Nicastrin
NEP	neprilysin
NFTs	neurofibrillary tangles
NGF	nerve growth factor
NMDA	*N*-methyl *D*-aspartate
NMDAR	NMDA receptor
NPCs	neural precursor cells
OXPHOS	oxidative phosphorylation

PDAPP	platelet-derived growth factor promoter expressing amyloid precursor protein
PCCs	posterior cingulate cortices
PDHC	pyruvate dehydrogenase complex
PEN2	prenisilin enhancer 2
POLG	proofreading-deficient mitochondrial DNA polymerase
PS1	prenisilin 1
PS2	prenisilin 2
RIP	regulated intramembrane proteolysis'
ROS	reactive oxygen species
RT-PCR	reverse transcriptase polymerase chain reaction
RMR	resting metabolic rate
SAPK	stress-activated protein kinase
SOD	superoxide dismutase
TDEE	total daily energy expenditure
Tg mAPP/ABAD	transgenic mutant APP and ABAD
Tg mAPP	transgenic mutant APP
TOM	translocase of the outer membrane machinery
VEGF	vascular endothelial growth factor
VO_2	rate of oxygen consumption
UCPs	uncoupling proteins

Introduction

Alzheimer's disease (AD) is the most common form of dementia, comprising approximately 60 percent of all dementia cases and affects more than 15 million people worldwide (Graff-Radford and Woodruff, 2007). Alzheimer's disease can be divided into two forms; early-onset (often familial) AD and late-onset (mostly sporadic) AD. The most important risk factor for sporadic AD is aging, while individuals with familial AD often have mutations in amyloid-associated genes.

AD is clinically characterized by a progressive loss of cognitive abilities. Pathologically, it is defined by the appearance of senile plaques composed of amyloid-β (Aβ), neurofibrillary tangles (NFTs) and massive neuronal cell and synapse loss at specific predilection sites (Selkoe 2002).

At present, no effective cure is available for Alzheimer's disease (AD). However, in light of recent research, which has demonstrated a significant energy deficiency in the brains of individuals with AD, it has been proposed that stimulation of the brain will slow down brain aging and diminish the risk for Alzheimer's disease, a principle known as the 'use it or lose it' theory (Swaab, 1991). This notion, however, might seem to contradict the long-withstanding amyloid cascade hypothesis, which proposes that amyloid-β peptide plaques, found in individuals with AD, are the culprit of Alzheimer's disease. However, by referring to some of the most prominent literature in the field of AD, this review will present the notion that Aβ peptides are actually involved in AD, but in a soluble form that exacerbates the age-related mitochondrial dysfunction responsible for the energy deficiency in AD. This review will also discuss the cognitive and physiological benefits of physical exercise and an engaging lifestyle – two practical approaches of the 'use it or lose it' theory – and describe how key elements of the research discussed prior are involved in these benefits.

Chapter 1. Amyloid cascade hypothesis

Ever since Alois Alzheimer microscopically analyzed the post-morbid brain of a fifty-one year old patient with strange behavioral symptoms and short-term memory loss and discovered the presence of plaques and neurofibrillary tangles (NFT), the groundwork was set for the amyloid cascade hypothesis, later proposed in the early nineties. This hypothesis suggests that the toxic effects of insoluble plaques, formed due to excessive accumulation and aggregation of amyloid-β peptide (Aβ), initiate a downstream cascade that leads to "synaptic alterations, microglial and astrocytic activation, the modification of the normally soluble tau protein into oligomers and then into insoluble neurofibrillary tangles, reduction in neurotransmitters and progressive neuronal loss" (Haass and Selkoe, 2007; Hardy and Higgins, 1992) resulting in the symptoms of Alzheimer's disease.

The amyloid cascade hypothesis has received a wealth of support over the years, but as many followers there are, equal amounts of contesters exist. This chapter will present the current debate of the amyloid cascade hypothesis by discussing primarily recent literature either supporting or contesting the notion, which should clarify the position of the 'use it or lose it' hypothesis in relation to it. Thereafter, the extent to which both theories can be tied together will be gauged.

i. Genetic studies provide evidence for amyloid cascade hypothesis

Genetic studies of familial Alzheimer's disease have served as the strongest foundation for the amyloid hypothesis, showing that mutations or polymorphisms in amyloid precursor protein (APP) or in genes that are directly involved in proteolytic processing of Aβ occur in all subjects with early-onset (familial) AD (Tanzi and Bertram, 2005). In 355 families, more than 160 fully penetrant autosomal-dominant mutations have been found on genes for the amyloid precursor protein on chromosome 21, prenisilin 1 (PS1) on chromosome 14, and prenisilin 2 (PS2) on chromosome 1 (Hellström-Lindahl et al., 2009). Even though these mutations occur in different genes located on different chromosomes, they all confer a biochemical pathway leading to the modified production of Aβ from amyloid precursor protein.

ii. Aβ production from amyloid precursor protein

Amyloid precursor protein, a membrane-anchored receptor, is processed by two different pathways. One involves prenisilin 1 and 2 and is amyloidogenic, i.e, yields Aβ, and the other produces non-amyloidogenic APP (forms an APP intracellular domain). The generation of Aβ from ubiquitously expressed APP has been thought to have a natural biological function, as cells are known to contain enzymatic machinery necessary for the production and degradation of Aβ (Crouch et al., 2008). However, the non-pathological role for Aβ is yet to be determined, although various studies have shown Aβ/APP to be involved in a broad range of cellular processes. The majority of APP is processed by the non-amyloidogenic pathway, which involves α-secretase cleavage of the peptide. In amyloidogenesis *(figure 1)*, on the other hand, APP is first cleaved in a general physiological mechanism known as 'regulated intramembrane proteolysis' (RIP) by β-site APP cleaving enzyme 1 (BACE1), a.k.a. β-secretase, resulting in the secretion of the large ectodomain. The remaining stub then binds to the active site of γ-secretase, which contains transmembrane domains presenilin-1 or 2. These domains bind to each other as well as three other proteins, anterior pharynx defective 1 (APH1a) (or APH1b), presenilin enhancer 2 (PEN2) and nicastrin (NCT), forming a core complex required for γ-secretase activity. Cleavage by the enzyme occurs in the middle of the membrane and results in the liberation of amyloid β-protein (Aβ) and the APP intracellular domain (AICD), of which the latter is only known to possess transcriptional regulatory activity (Zhang et al., 2007). γ-secretase can cleave the protein at several sites after amino acids 38, 40 or 42, and therefore has the propensity to yield three different isoforms, determining the peptide's toxicity. The most common isoforms are Aβ40 and Aβ42, with Aβ40 being the most frequently cleaved, but Aβ42 is the most fibrillogenic and

thus has a greater tendency to aggregate and form the amyloid plaques, and is thus associated with Alzheimer's disease (Wilkett and De Strooper, 2004; Haass and Selkoe, 2007).

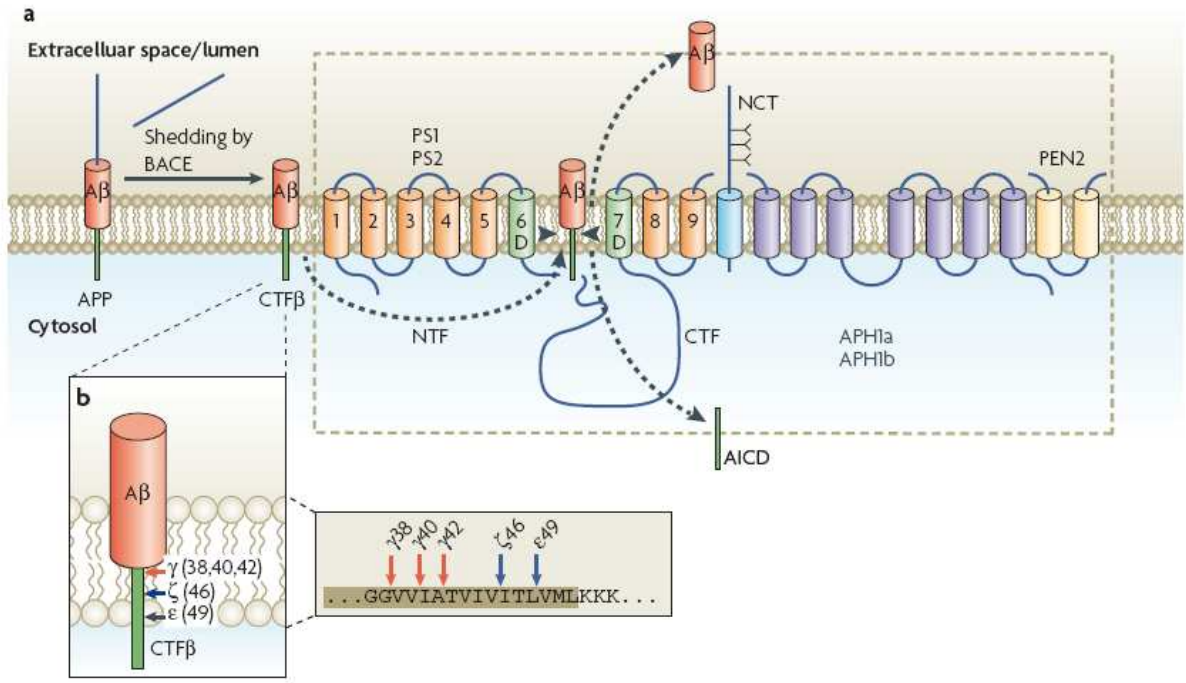

Figure 1. **Production of amyloid-β protein from amyloid precursor protein by the amyloidogenic pathway**.
a. APP is cleaved by β-secretase, resulting in the secretion of the large ectodomain.The remaining stub then binds to the active site of γ-secretase, which contains transmembrane domains presenilin-1 or 2. These domains bind to each other and three other proteins, anterior pharynx defective 1 (APH1a) (or APH1b), presenilin enhancer 2 (PEN2) and nicastrin (NCT), forming a core complex required for γ-secretase activity. Cleavage by the enzyme occurs in the middle of the membrane and results in the liberation of amyloid β-protein (Aβ) and the APP intracellular domain (AICD).
b. γ-secretase can cleave the protein intramembranously at several sites after amino acids 38, 40 or 42, and therefore has the propensity to yield three different isoforms, determining the peptide's toxicity (the numbers in the image refer to the sequence of Aβ, whereas the shaded amino acids represent the region in the transmembrane domain).
(Image taken from Haass and Selkoe, 2007).

Point mutations in APP, PS1 and PS2 appear to accelerate amyloid plaque formation by increasing the production of cerebral Aβ42 compared to Aβ40 (Tanzi and Bertram, 2005). As suggested by the amyloid hypothesis, all identified APP mutations associated with AD occur either within or along the Aβ region of this polypeptide. In line with this, the mutations that flank the Aβ region near the β-secretase site augment β-secretase cleavage and increase general Aβ levels, whereas "the mutations within the Aβ region near the γ-secretase site specifically enhance the oligomerization of the peptide by increasing the production of the highly amyloidogenic Aβ42 isoform" (Haass and Selkoe, 2007; Citron et al., 1992; Cai et al., 1993). In a similar fashion, studies on mutant prenisilins demonstrated an enhanced amyloidogenic processing of the amyloid precursor protein by a selective increase in the production of Aβ42 (Citron et al., 1998). At this point, however, it is unclear whether these mutations represent a toxic gain-of-function or a loss-of-function of APP and prenisilins (De Strooper, 2007; Hardy, 2007; Van Broeck et al., 2007; Wolfe, 2007).

Neverthelesss, the fact that an increase in total Aβ load can accelerate Aβ deposition has also been demonstrated by the neuropathology in patients with Down syndrome, a disorder caused by trisomy of chromosome 21, on which APP is localized (Glenner and Wong, 1984a; Rovelet-Lecrux et al., 2006; Sleegers et al., 2006). Overexpression of AβPP, as a result of an additional copy of APP being present, appears to cause the formation of Aβ plaques similar to those seen in AD patients, which clarifies why patients with Down syndrome are often found to develop Alzheimer's disease in their forties. Clearly, the fact that all carriers of this mutation develop familial AD suggests a strong causative connection between amyloid and Alzheimer's disease.

iii. Evidence from *in vivo* mouse models

Our understanding of the molecular and cellular processes involved in AD has been markedly enhanced by mouse models, especially in terms of understanding mechanisms relating to Aβ production, deposition and clearance, and helped develop immune and non-immune therapeutic strategies for AD. Mice models are particularly ideal for studying aging as they have a relatively short life span of approximately 30 months. Not only that, mice have the capacity to perform a number of spatial and associative learning and memory tasks, many of which are hippocampus-dependent and relatively comparable to cognitive functions impaired in humans with AD.

Many groups have generated mouse models of fAD by overexpressing fAD mutant APP or PS1 or both. Double transgenic AβPP+PS1 mutated mice demonstrate plaques rapidly and progressively developing in their brain, starting at 3 months of age and becoming stable at 12 months (Holcomb et al., 1998). Not only that, these mice are also impaired in spatial learning tasks by the age of 15 months (Arendash et al., 2001; Gordon et al., 2001). In some cases, memory function improved in unison with a reduction in total Aβ or Aβ plaque load in behavioral paradigms (Radde et al., 2008), but on the whole, many mouse models of AD show memory deficits long before the plaques are observed in the brain (Lesne et al., 2008). The deleterious events observed as a result of AβPP and PS1 overexpression were also found *in vivo* when fibrillar Aβ was injected in mouse brains.

The core limitation of the AβPP + PS1 AD mouse model, however, is the fact that it serves more as a model for amyloidosis than AD as a whole, as the mutations cause little neurofibrillary tangle formation or 'global' neuronal loss in the mouse brains (Morgan and Keller, 2002). Only in mice with prominent neuritic plaques has amyloid burden been found to correlate with a region-specific neuron loss (Calhoun, 1998). In APP and APP/PS transgenic mice, abnormal tau phosphorylation patterns were observed only in the vicinity of amyloid plaques (Kurt et al., 2003; Radde et al., 2006). Nevertheless, the mechanistic interplay between Aβ and tau has been quite convincingly demonstrated by the cross-breeding of mice overexpressing mutant tau with mice overexpressing mutant APP (Lewis et al., 2001; Oddo et al., 2003; Bolmont et al., 2007). In a similar fashion, "injection of synthetic Aβ42 fibrils or small amounts of Aβ-containing brain extract into the brains of P301L mutant tau transgenic mice was shown to accelerate tangle formation" (Radde et al., 2008). However, this still does not explain why NFT are not found beyond the vicinity of amyloid plaques, as in AD-affected humans. Thus, in spite of significant advances made with the initial transgenic mouse models of Alzheimer's disease, there is a general agreement that these models fail to induce the entire pathological cascade of human AD neuropathology in mice.

Even if the mouse models were all-encompassing, the mice would not provide an accurate model for sporadic, age-related Alzheimer's disease, representing the majority of AD cases. As amyloid deposition starts much earlier in mice due to hyperexpression of the fAD genes, age-related factors are unable to provide the additional dose found to be the greatest risk factor for sporadic AD.

iv. Evidence from *in vitro* tissue culture studies

However, perhaps tissue culture studies *in vitro* provide some support to the amyloid cascade hypothesis, as they demonstrated that amyloid-β peptides have a neurotoxic nature. To be specific, a number of early studies showed that when added to neuronal cell culture, fibrillar Aβ had detrimental effects such as apoptosis, neuronal cell death, and synaptic and dendritic loss. However, it has been suggested that these effects may in fact be an artifact of culture and not intrinsic to the peptide itself (Rottkamp et al., 2001).

v. Evidence from human studies

Moreover, when humans were scanned for amyloid burden, the plaque load did not correlate well with the degree of dementia in humans (Terry et al., 1991) and many AD patients with severely impaired memory show no plaques at post-mortem analysis (Pimplikar, 2009). Conversely, many healthy aging individuals have amyloid plaques in their brains, suggesting that amyloid production may partly be caused by cellular stress, as a result of aging.

In sum, even though the cell culture, mice and human studies have proved to be valuable in investigating particular aspects of Alzheimer's disease, on the whole, the data does not seem to provide a solid foundation for the amyloid cascade hypothesis.

2. Soluble amyloid-β: the hidden culprit

However, before throwing Aβ out with the bathwater completely, a surprising compromise may, in fact, lie in the wealth of literature demonstrating a relationship between *soluble* low-weight Aβ and Alzheimer's disease. As was mentioned before, increasing evidence has shown that fibrillar amyloid plaques are unrelated to Alzheimer's disease. This is not surprising, as not only were these earlier studies conducted at a time when Aβ assemblies formed *in vitro* were poorly characterized, they were also based on the assumption that since amyloid fibrils were detectable, they must have been the only toxic moiety of Alzheimer's disease. However, as neither fibrillar nor diffuse Aβ monomers were found to be correlated with cognitive abnormalities, researchers began to speculate whether another, more soluble form of Aβ could be responsible. After all, it would only make sense for an Aβ species that can actually diffuse and enter the spaces in and surrounding neural synapses to be responsible for the synaptic and neuronal dysfunction occuring at locations distant from plaques.

Fibrillization of Aβ is preceded by multiple conformational changes including trimer, pentamer, or higher molecular weight complex formation, also known as Aβ-derived diffusible ligands (ADDLs) (Lambert et al., 1998), oligomers composed of 15–20 monomers (AβOs) (Kayed et al., 2003), protofibrils (string of oligomers) (Nguyen and Hall, 2004), and dodecameric oligomers Aβ*56 (Lesné et al., 2006). These non-fibrillar, intermediate Aβ species are what researchers collectively refer to as "soluble Aβ" (Glabe, 2004). The exact constitution of soluble Aβ, however, is still not well-understood. The term soluble Aβ is an "operational definition" (Shankar and Walsh, 2009), encompassing all forms of Aβ "that remain in aqueous solution following high speed centrifugation of brain extracts" (Shankar and Walsh, 2009; McLean et al., 1999; Lue et al., 1999; Wang et al., 1999). As extraction of Aβ from the brain consistently involves homogenization and subsequent cell fracture, the extracted solution will include "truly soluble extracellular Aβ, extracellular Aβ loosely associated or in equilibrium with plaques and a portion of intracellular Aβ" (Shankar and Walsh, 2009).

To date, most studies of soluble cerebral Aβ have used enzyme-linked immunosorbent assay (ELISA) methods that were unable to reveal the aggregation state of the species detected and seemed to have a preference for detecting Aβ monomer (Stenh et al., 2005; Morishima-Kawashina and Ihara, 1998; Enya et al., 1999; Funato et al., 1999 and Englund et al., 2009). In spite of this, "the fact that Aβ is not sedimented by ultracentrifugation suggests that they are not mature amyloid fibrils" (Shankar and Walsh, 2009). Moreover, although a wealth of literature exists on the primary sequence of cerebral Aβ, only limited data exists on the assembly forms of Aβ present in human brain. Using aqueous buffer free of detergents or chaotropes, i.e. denaturing agents, Kuo and colleagues (1996) isolated a range of non-fibrillar forms of Aβ from both AD and control brain. "Both extracts contained a continuous distribution of Aβ species ranging from monomers up to oligomers as large as 100 kDa, with low-n oligomers from dimer to octamers making the major contribution" (Shankar and Walsh, 2009). Higher molecular weight assemblies have not been found in human cerebral spinal fluid (CSF) or soluble human brain extracts. However, whether the lower-weight species of Aβ are true forms of Aβ oligomers or are breakdown products of larger-weight assemblies is uncertain (Shankar and Walsh, 2009). Nevertheless, the presence of dimers and trimers in the soluble fraction of human brain and in amyloid plaque extracts (McLean et al., 1999; Enya et al., 1999; Funato et al., 1999) suggests that apart from mediating neuronal dysfunction, low-n oligomers also aggregate to form the insoluble amyloid deposits.

Biochemical analyses of AD brain in recent years have found the CSF soluble Aβ levels to correlate strongly with the extent of synaptic loss and degree of cognitive impairment (Shankar and Walsh, 2009; Lemere et al., 2002; McLean et al., 1999; Lue et al., 1999 and Wang et al., 1999). Most importantly, "the biochemically measured levels of soluble Aβ oligomers were shown to correlate much better with the presence and degree of cognitive deficits than simple plaque counts" (Haass and Selkoe, 2007; Naslund et al., 2000; Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). This evidence coupled with the fact that the Aβ surface area of large fibrillar plaques presented to neuronal membranes is significantly smaller than that of lower-weight, soluble oligomers, capable of diffusing

into synaptic clefts and the space surrounding it, indicates that soluble Aβ may be responsible for the neuronal and/or synaptic dysfunction associated with impaired memory in AD.

However, before dismissing fibrillar Aβ as a toxic moiety completely, it was mentioned before that there are several reports of the fibrillar form of Aβ having modest deleterious effects in mice and cell culture. These results are still considered to be viable when one simply thinks of soluble Aβ being *more* toxic than fibrillar Aβ instead. This was demonstrated by Deshpande and colleagues (2006) when they investigated the effects of three distinct assembly forms of Aβ, namely high molecular weight oligomers, ADDLs and fibrillar Aβ. Their study demonstrated that small concentrations (~5 μM) of high molecular weight synthetic oligomers caused global neuron loss within 24 hours, whereas the time required for the same concentration of ADDLs to acquire the same effect took 5-times as long, and fibrillar Aβ was only able to induce modest cell death after 10 days when the concentration was 4-fold higher. So undoubtedly, as mouse and cell culture studies already demonstrated, fibrillar Aβ has toxic effects on neurons, but is simply not harmful enough on its own to cause Alzheimer's disease and the associated defects.

Noteworthy evidence supporting the fact that soluble forms of amyloid-β act as the principal mediators of neuronal dysfunction, comes from a study that showed that a single intraperitoneal injection of an anti-Aβ antibody could reverse deficits of memory in platelet-derived growth factor promoter expressing amyloid precursor protein (PDAPP) mice (Dodart, 2002). At the same time, however, brain amyloid burden did not decrease in these mice, suggesting that the antibody reacted with the soluble, diffusible species of Aβ and that the resultant removal or neutralization of these peptides must have caused the swift improvement in object recognition performance. In line with this is a study conducted by Lesné and colleagues (2008) on APP transgenic (Tg2576) mice with plaques but markedly reduced levels of Aβ oligomers, providing them the opportunity to study the effects of plaques on memory alone. These mice were found to have normal functioning memory during a period of decreased Aβ oligomers levels, but rapid amyloid plaque formation. This not only hints at the importance of Aβ oligomers in memory loss, but also confirms that amyloid plaques are not the initial culprit of memory impairment.

A large number of studies have demonstrated the specific deleterious effects of soluble Aβ on synaptic function in rodents. First of all, Selkoe (2008) found specific APP-expressing cultured cell lines that are able to synthesize low-n oligomers intracellularly and release a fraction of these short amino acid chains into the medium. In addition, they demonstrated that low (picomolar) concentrations of these soluble oligomers could "disrupt long-term potentiation (LTP) in hippocampal slices and *in vivo*, and impair the memory of a complex learned behavior in rats". In line with this, Shankar and colleagues (2008) show that soluble Aβ "isolated directly from AD brains potently and consistently induces several AD-like phenotypes in normal adult rodents," namely reduced dendritic spine density, inhibition of LTP and facilitation of long-term depression (LTD) in the hippocampus, and affected memory of a learned behaviour.

Activation of NMDA (*N*-methyl *D*-aspartate) receptors and/or metabotropic glutamate receptors (mGluR) are required for the induction of hippocampal synapse potentiation and depression, depending on the experimental conditions (Kemp and Bashir, 2001; Citri and Malenka, 2008). Mechanistically, the balance between LTP versus LTD is thought to rely on changes in cytosolic Ca2+ concentration and the differential activation of particular phosphatases and kinases, such as calcium/calmodulin dependent kinase II (CamKII), calcineurin, and cyclic AMP response element binding protein (CREB) (Kemp and Bashir, 2001; Citri and Malenka, 2008). Ultimately, this balance in neuronal signalling was suggested to control the "stability of the post-synaptic density" (Shankar and Walsh, 2009; Colbran, 2004) and "the post-synaptic content of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors" (Shankar and Walsh, 2009; Kessels et al., 2009; Kessels and Malinow, 2009). In turn, these structural changes regulate the magnitude and dynamics of neurotransmission at the synaptic level.

A recent study conducted by Selkoe (2008) on natural soluble oligomers suggests a model in which exposure to Aβ oligomers results in "partial NMDA receptor (NMDAR) blockade, either by reducing NMDAR activation, reducing NMDAR-dependent calcium influx, or enhancing NMDAR-dependent activation of calcineurin". Selkoe proposes that these Aβ-mediated changes "promote the LTD-inducing mode and inhibit the LTP-inducing mode of NMDAR-dependent signalling" (Selkoe, 2008). Since LTP induction results in spine enlargement and growth, whereas LTD induction causes spines to shrink and retract (Matsuzaki, 2004; Nagerl et al., 2004; Zhou et al., 2004), this Aβ oligomer-induced imbalance can be said to cause progressive loss of dendritic spines and glutamatergic synapses (Selkoe, 2008). As synaptic plasticity is intricately involved in neural viability, it therefore seems more than logical that persistant disruption of synaptic plasticity by soluble Aβ would induce synaptic loss, especially considering the fact that synapse loss is an early and signifying characteristic of AD and is known to correlate with the degree of cognitive impairment in AD subjects.

The inhibitory effect of soluble Aβ on long-term potentiation has provided a model system for studying potential therapies, which, in turn, has provided data confirming the role of soluble Aβ in the pathology of AD. To begin with, a therapeutic study by Klyubin and colleagues (2005) demonstrated that injecting an Aβ monoclonal antibody shortly following the injection of naturally secreted human Aβ completely prevented the inhibition of LTP. Importantly, endogenous antibodies raised by Aβ vaccination also averted oligomer-mediated LTP inhibition in rats. In fact, the level of circulating Aβ oligomer-recognizing antibodies roughly correlated with the degree of protection. Similarly, one study showed that Aβ oligomer-induced spine loss is thwarted by Aβ antibodies and a small molecular inhibitor of Aβ aggregation, whereas another showed that dendritic spine density in organotypic hippocampal slice cultures was reduced by cell-derived Aβ oligomers (Selkoe, 2008). Administration of Aβ antibodies or small-molecular modulators of Aβ aggregation, however, were able to avert these changes. Clearly, this is all relevant in light of recent studies suggesting that therapeutically lowering cortical Aβ levels in some AD patients could actually be responsible for stabilization of memory and cognitive decline (Hock et al. 2003; Nicoll et al. 2003; Gilman et al. 2004).

The only gene that has been unequivocally linked to the prevalent late-onset form of Alzheimer's disease is apolipoprotein E (apoE) (Strittmatter et al., 1993). Where the E-2 allele has been thought to protect against the development of AD, the E-4 variant of this gene, was shown to pose a significant risk for developing AD, increasing the chance by as much as 10–12-fold when present in the homozygous state (Corder et al., 1993). Individuals with an apoE4 allele were shown to suffer increased Aβ deposition in the brain, whereas APP transgenic mice experienced enhanced Aβ fibrillization (Holtzman et al., 2000). The mechanism by which apoE4 increases the risk of Alzheimer's disease remains an active area of investigation, but evidence has shown that the apoE4 genotype is associated with lower neuronal metabolic activity in the nucleus basalis of Meynert in AD patients and controls, as indicated by the size of the Golgi apparatus, a sensitive parameter for changes in neuronal metabolic activity (Dubelaar et al., 2004). However, this notion of reduced metabolic activity will be elaborated on in the next chapter.

Altogether, the data appear to argue in favour of the involvement of soluble Aβ in Alzheimer's disease, as this soluble peptide was shown to interfere with synaptic structure and function. Hence, where evidence of a link between the amyloid hypothesis and Alzheimer's disease was initially lacking in the previous chapter, it now clearly seems to support a relationship between the hypothesis and the 'use it or lose it' theory. However, this is not the complete picture of AD etiology, as several other factors appear to contribute significantly to the disease. In fact, these factors can also be traced back to the neuronal activation hypothesis, one of them being mitochondrial dysfunction due to aging, which will be discussed in the next chapter.

3. Impaired mitochondrial function and energy deficiency in AD

As Swaab and colleagues postulate in the 'use it or lose it' review, decreased neuronal activity is an essential characteristic of AD, either as a risk factor or direct pathogenic aspect. Although a basis of this decreased activity was shown to be due to soluble Aβ-induced synaptic dysfunction, a significant number of studies have found glucose utilization to be reduced and mitochondrial function to be impaired in both aging and AD. Thus, impaired mitochondrial function may be one possible mechanism responsible for the energy deficiency in AD (Blass et al., 2000; Mosconi et al., 2005; Atamna and Frey, 2007; Parihar and Brewer, 2007) and could also be a determining factor of synaptic dysfunction in AD (Mosconi et al., 2008).

Normal synapse function requires concerted activity of a multitude of mechanisms, including the generation of gene products involved in membrane complex formation and maintenance; mRNA, protein, and neurotransmitter synthesis and delivery; and most importantly, the maintenance of ion gradients across the plasma membranes, as they are critical for the generation of action potentials (Costantini et al., 2008). However, these actions can only be performed efficiently when sufficient energetic substrates are supplied. Glucose is the primary fuel for the brain under normal circumstances, with fatty acids only making a minor contribution. Glucose metabolism is the most important function of mitochondria in all types of cells (Tyler, 1992) and the human brain one of the most metabolically active organs in the body, requiring large amounts of energy to function properly. This intense demand for energy is incessant; even brief periods of oxygen (O_2) or glucose deprivation result in neuronal death. However, in spite of this high energy requirement, the brain is rather uncompromising in its ability to utilize substrates for energy production. The free energy necessary to drive most cellular reactions is derived from phosphorylation of adenosine -5'-triphosphate (ATP), which is mostly produced in the mitochondria by the oxidation of glucose under aerobic conditions (Atamna and Frey, 2007).

During normal neuronal function, glucose is metabolized through the glycolytic pathway to pyruvic acid in the cytoplasm, yielding two ATP molecules, after which pyruvate is oxidized to acetyl co-enzyme A (acetyl-CoA) and carbon dioxide (CO_2) by the pyruvate dehydrogenase complex during the link-reaction in the mitochondria (Schubert, 2005). Once acetyl-CoA is formed, either aerobic or anaerobic respiration will occur. When oxygen is present, aerobic respiration will occur in the mitochondria, leading to the Krebs cycle. In the absence of oxygen, fermentation of the pyruvate molecule will occur. After acetyl-CoA production (in the presence of oxygen), the molecule will enter the Krebs cycle inside the mitochondrial matrix where it is broken down into different substances yielding free electrons and CO_2. The electrons are, in turn, accepted by nicotinamide adenine dinucleotide (NAD), reducing it to NADH, and are carried to the electron transport chain (ETC). The electron transport complexes (I, II, III, and IV) and the Krebs cycle, which together make up the process termed oxidative phosphorylation (OXPHOS), are the mitochondrial metabolic pathways that are essential for generating the proton gradient across the inner membrane of the mitochondria that is used to produce ATP by phosphorylation of adenosine diphosphate (ADP) by ATP synthase, and in turn, the energy needed to fuel neural activity. The electron transport complexes require cardiolipin, coenzyme Q, copper, heme, and iron-sulfur clusters for proper functioning. The Krebs cycle extracts the electrons from the intermediate metabolites to reduce NAD and flavin adenine dinucleotide (FAD). The reduced NAD and FAD are the major source of electrons for the ETC and pass the electrons to complexes I and II, respectively. Finally, the electrons are transferred to exogenous oxygen, which, in turn, is reduced to produce water (Atamna and Frey, 2007).

Although electron transport occurs with great efficiency, a small fraction of electrons are prematurely leaked to O_2, resulting in its incomplete reduction to the toxic free-radical superoxide. Superoxide is not particularly reactive by itself, but can damage lipids and proteins in its hydroperoxyl ($HO_2\cdot$) form, known as oxidative stress. These chemically-reactive, O_2-containing molecules are known as reactive oxygen species (ROS). In normal conditions, antioxidant defenses counteract ROS. In particular,

antioxidants catalase and superoxide dismutase (SOD) help to minimize the damaging effects of hydrogen peroxide (H_2O_2) by converting it into benign O_2 and water molecules (Moreira, 2009).

Keeping with the notion of reduced neuronal energy, a vast amount of evidence suggests that altered glucose metabolism is a very early change in AD (de Leon et al., 2001; Mosconi et al., 2006, 2008; Small et al., 1995; Reiman et al., 1996) and is an excellent correlate of the clinical disabilities in dementia (Blass, 2002; Mosconi et al., 2008). In fact, disturbances in the cerebral metabolic rate of glucose (CMRglu) have been observed in patients with Alzheimer's disease as early as 1983, when de Leon and coworkers (1983) examined 24 elderly patients (with an average age of 73 years) with senile dementia and noted reductions of 17 to 24 percent in regional CMRglu. In turn, these reductions in glucose utilization linked to cognitive performance, suggesting "that such declines can be used as a reliable marker of disease status" (Constantini et al., 2008). Practically all fludeoxyglucose-Positron Emission Tomography (FDG–PET) studies report that, compared with age-matched healthy normal controls, AD patients have regional metabolic reductions in the parieto-temporal (Friedland et al., 1983) and posterior cingulate cortices (PCCs) (Minoshima et al., 1997) and the frontal areas in the advanced state of AD (Foster et al., 1984). These reductions are part of a widespread global metabolic impairment (Ferris et al., 1980), although in general, the primary motor and visual areas, cerebellum, thalamus, and basal ganglia nuclei are relatively spared (Mazziotta and Phelps, 1986). On the whole, these findings have been consistently reproduced by studies conducted since the early 1980s, and this pattern of hypometabolism is therefore now widely accepted as a reliable *in vivo* hallmark of Alzheimer's disease, and accurately distinguishes AD from normal aging.

Such defect(s) in glucose utilization suggest possible abnormalities in mitochondrial function in Alzheimer's disease. In support of this concept, a range of reports have shown altered mitochondrial properties in Alzheimer's disease, in particular, the energy extracting mechanisms of the mitochondria. *In vitro* analyses of AD autopsy brain studies have shown that the most consistent defect in mitochondria in AD are the reductions in Vmax activities of several key enzymes responsible for oxidative metabolism, including α-ketoglutarate dehydrogenase complex (α-KGDHC) and pyruvate dehydrogenase complex (PDHC), two enzymes involved in the rate-limiting step of tricarboxylic acid cycle (Parker et al., 1994; Gibson et al., 1998; Nagy et al., 1999; Swerdlow and Kish, 2002). In addition, studies have shown reduced cytochrome oxidase (COX) activity, the terminal enzyme in the ETC that is responsible for reducing molecular oxygen (Parker et al., 1994; Gibson et al., 1998; Maurer et al., 2000; Bosetti et al., 2002; Swerdlow and Kish, 2002). Studies using different experimental set-ups have suggested that COX activity in AD subjects is 10–50 percent less than in age-matched controls (Swedlow, 2009). And as this reduction in cytochrome oxidase has been shown to occur in platelets, fibroblasts, and large parts of the brain (Swerdlow and Kish, 2002), it is therefore not an artefact of neurodegeneration. Furthermore, since COX is an intracellular measure of oxidative energy metabolic capacity and respiration (Villani and Attardi, 2000), it therefore appears to be a significant molecular candidate as a contributor to the energy hypometabolism experienced in AD.

At this point, however, it is unclear to what extent Vmax reduction reflects reduced COX enzyme expression or a structural change in the enzyme. Numerous studies have linked abnormal mitochondrial protein function to the altered neuronal expression of nuclear and mitochondrial genes encoding subunits of the mitochondrial ETC. Liang and collaborators (2008) showed that compared to controls, AD cases had the largest proportion (70 percent) of underexpressed genes comprising the nuclear genes encoding for subunits of the mitochondrial ETC complexes and translocases of the inner and outer mitochondrial membranes (TIMMs and TOMMs respectively) in the posterior cingulate cortex, a brain region which PET studies found to be metabolically affected in the earliest stages of AD. The visual cortex, on the other hand, a brain region relatively spared in AD, contained significantly less underexpressed genes than the PCC (*table 1*).

Brain region	Complex I		Complex II		Complex III		Complex IV		Complex V		TIMMs		TOMMs	
	No. of subunits	Fold	No. of subunits	Fold	No. of subunits	Fold	No. of subunits	Fold	No. of subunits	Fold	No. of subunits	Fold	No. of subunits	Fold
PCC	28/39(72)	−3.0	2/4(50)	−2.8	8/9(89)	−3.6	7/13(54)	−2.8	11/15(73)	−3.8	3/11(27)	−3.2	3/6(50)	−2.6
MTG	27/39(69)	−2.5	2/4(50)	−2.5	6/9(67)	−2.3	8/13(62)	−2.4	9/15(60)	−3.3	3/11(27)	−2.2	5/6(83)	−2.3
HIP	25/39(64)	−2.5	2/4(50)	−2.7	5/9(56)	−2.8	7/13(54)	−2.2	10/15(67)	−2.7	3/11(27)	−2.6	4/6(67)	−2.7
EC	9/39(23)	−4.5	0/4 (0)		1/9(11)	−3.7	3/13(23)	−4.5	5/15(33)	−4.3	5/11(45)	−3.7	1/6(17)	−2.8
VC	7/39(18)	−1.8	0/4 (0)		1/9(11)	−2.0	1/13 (8)	−1.5	4/15(27)	−2.1	0/11 (0)		1/6(17)	−2.2
SFG	4/39(10)	−3.9	0/4 (0)		0/9 (0)		0/13 (0)		0/15 (0)		2/11(18)	−3.6	2/6(33)	−2.4

Table 1. The proportion of underexpressed mitochondrial-metabolism related genes in 6 sample brain regions. The numerator of the different values for the No. of subunits represents the number of subunits showing statistically significant ($P < 0.01$ with multiple testing corrections) underexpression whereas the denominator indicates the total number of nuclear-encoded subunits for the complex or translocase. The value in brackets is this ratio expressed as a percentrage. PCC = posterior cingulated cortex; MTG = middle temporal gyrus; HIP = hippocampus; EC = enthorinal cortex; VC = visual cortex; SFG = superior frontal gyrus. (Table and corresponding information taken from Liang et al., 2008).

In line with this, using reverse transcriptase polymerase chain reaction (RT-PCR) techniques to study mRNA expression of 11 mitochondrial-encoded genes in patients with early AD and with definite AD, as well as age-matched control subjects, Reddy and Beal (2008) found mitochondrial genes in complex I of OXPHOS to be downregulated in both early and definite AD brain specimens, whereas complexes III and IV (COX) showed increased mtRNA expressions. Several other studies have also reported this phenomenon (Hirai et al. 2001; Strazielle et al. 2003; Manczak et al. 2004; Reddy et al. 2004), which could be interpreted as a compensatory mechanism for the decreased COX function and the consequent increase in demand on energy production. As spectral analysis of the enzyme has indicated that COX is kinetically altered in AD and lacks one of its two substrate binding sites (Parker and Parks, 1995), altogether the data would imply that COX activity is reduced not because the quantity is decreased as a result of down-regulated gene expression, but because in AD, the enzyme is structurally different from that in controls.

However, other studies have shown that cytochrome oxidase activity is reduced as a result of decreased mitochondrial RNA (mtRNA) expression. Several studies demonstrated decreased mtRNA expression in complexes I and IV (Chandrasekaran et al. 1994, 1996, 1997; Simonian and Hyman, 1994) and decreased mRNA expression of nuclear-encoded mitochondrial genes in complexes IV and V in brains of AD patients (Chandrasekaran et al. 1994, 1997; Simonian and Hyman 1994; Kish et al., 1999). Clearly, at this point, it cannot be said whether reduced mtRNA expression, altered enzyme kinetics or both, contribute to reduced COX function in AD. Some studies suggest that mitochondrial DNA (mtDNA) and mitochondrial number increase in AD, which would indicate altered COX kinetics, while others insinuate that mtDNA and mitochondrial number decline, proposing reduced COX expression (de la Monte et al., 2000; Hirai et al., 2001; Baloyannis, 2006). Thus, it can only be noted that as far as bioenergetics goes this debate does not stop with COX.

Cybrid studies suggest that mtDNA is at least partly responsible for reduced COX activity in AD (Swerdlow et al., 1997; Swerdlow, 2007c). Cybrids are cell lines created by placing mitochondria from individual human subjects into cultured cells (King and Attardi, 1989). The studies show that mitochondria derived from platelets of individuals with AD have decreased COX activity (Swerdlow, 2007c). This specific defect continues over time in the cybrid lines, which implies that mtDNA differs between AD and control subjects and that these differences can change COX. Although specific sequence-level differences between AD and control subject brain and platelet mtDNA have been observed in some studies, it is unclear whether these differences are also present in cybrids (Corral-Debrinski et al., 1994; Mecocci et al., 1994; Coskun et al., 2004; Coon et al., 2006).

Data from epidemiologic, neuropsychological, biomarker and cell biology studies suggest that the cause of these mtDNA defects is due to mitochondrial inheritance. One study reported that mtDNA haplogroups influence AD risk (van der Walt et al., 2004), whereas another found that for AD subjects

with a demented parent, the demented parent was more often the mother (Edland et al., 1996). This relationship persisted after greater female longevity was corrected for and suggests that having an AD mother bestows a higher risk of developing Alzheimer's disease than having an AD father. A large analysis of non-demented, middle aged offspring found that individuals with an AD-affected mother performed worse on neuropsychological tests than those with an AD-affected father or no AD affected parent (Wolf et al., 2005). This could reflect a presymptomatic AD stage, as cognitively sound, middle aged individuals with AD mothers but not AD fathers also show CMRglu reduction patterns characteristic of AD (Mosconi et al., 2007). Furthermore, it has also been reported that COX activity is lower in cybrid cell lines containing mtDNA from persons with AD mothers than those containing mtDNA from individuals with AD fathers (Davis et al., 1997). All in all, the data therefore suggest that mitochondrial inheritance could influence AD risk and pathology.

However, genomic inheritance is unlikely to be the sole contributor to mitochondrial protein dysfunction in AD. As a matter of fact, both nuclear DNA (Kirkwood and Austas, 2000) and mtDNA undergo age-related mutational changes or damage, which correlate with impaired mitochondrial function. Mitochondrial DNA is distinct from and replicates independently of nuclear DNA and mutates at higher rates than nuclear DNA. As it contains few introns and primarily encodes either polypeptides of the ETC or components required for their synthesis, it makes the rest of the molecule crucial for maintaining the oxidative phosphorylation process located on the inner membrane within mitochondria. Thus, any coding mutations in mtDNA will affect the electron transport chain as a whole, disturbing both the assembly and function of the products of numerous nuclear genes in electron transport chain complexes (Hiona and Leeuwenburgh, 2008). In turn, these ETC defects can have "pleiotropic effects" (Hiona and Leeuwenburgh, 2008), as the cellular energetics will be affected as a whole (Alexeyev et al., 2004). Nevertheless, each cell contains multiple copies of mtDNA to ensure that if a mutation does occur within the mitochondrial genome, it can exist among wild-type copies, a situation known as heteroplasmy. It is only when an mtDNA mutation reaches a certain threshold that a biochemical defect, such as a respiratory chain deficiency will become detectable (Krishnan et al., 2007).

Nevertheless, mtDNA mutations have been linked to an increased incidence of AD (Wallace et al. 1997; Coskun et al. 2004). Coskun and colleagues (2004) discovered that in 65 percent of all AD brains, the T414G mutation was present in the sequence of the mtDNA control region (CR), whereas none of the controls harboured this mutation. Moreover, by cloning and sequencing the mtDNA CR from AD and control brains, all AD brains were found to have an average of 63 percent increase in heteroplasmic mtDNA CR mutations, whereas AD brains specifically from subjects 80 years and older had an even greater (130 percent) increase in heteroplasmic CR mutations. Not only that, these mutations were found to preferentially alter known mtDNA regulatory elements and have direct consequences on the binding of mitochondrial proteins to the genome, thus affecting maintenance, replication, transcription, and translation (Suissa et al. 2009). Hence, these mutations could also explain the average 50 percent reduction in the mtDNA L-strand ND6 transcript and the mtDNA/nuclear DNA ratio in AD patients (Coskun et al., 2004), which, in turn, would be expected to be responsible for a reduction in brain oxidative phosphorylation and the consequent mitochondrial defects observed in AD.

4. Mitochondrial theory of aging and Alzheimer's disease

Although the data indicate that one of the contributing factors to AD pathology is a surpassed threshold of mtDNA mutations, the question is, of course: what causes the accumulation of mutations towards the end of a person's lifespan to begin with? A long withstanding theory called the mitochondrial theory of aging (Harman, 1972) has attempted to explain this. This theory proposes that during the course of an individual's life, reactive oxygen species, produced by the ETC in mitochondria as natural by-products of oxidative phosphorylation, build up and modify cell components, known as oxidative stress (Harman, 1981). As the primary source of reactive oxygen species, mitochondria are said to be affected the most, leading to changes in their (DNA) structure and, in turn, to OXPHOS dysfunction (Balaban et al., 2005).

This gradual process of ROS accumulation and oxidative damage to mitochondria over time has been summarized in detail by de Grey (1999), who surmises that sufficient free radical damage to mtDNA terminates the oxidative phosphorylation process within that particular mitochondrion, as the required proteins cease to be produced. The mitochondrion will thereby change its energy extracting method to one that is more efficient and does not produce free radicals, but has to be employed at a greater rate to produce similar levels of ATP. Mitochondria, like most cellular components, are recycled on a regular basis by lysosomes, which engulf and break down impaired or worn-out mitochondria in response to sufficient damage to its membrane. The surviving mitochondria within a cell will then divide and replicate to compensate. However, if mitochondrial DNA has been damaged beyond the threshold at which OXPHOS occurs correctly, the mitochondrion will cease to produce membrane-damaging free radicals and thereby the cue to be broken down by a lysosome. As a result, when the mitochondrion divides and replicates thereafter, its damaged DNA will be replicated into new mitochondria. And as free radicals are not produced in these new mitochondria due to terminated OXPHOS, they will not be recycled by lysosomes.

In turn, one mitochondrion with damaged DNA and ceased OXPHOS function will ultimately replace a significant portion of a cell's entire mitochondrial population in this manner, with perhaps 1 percent of an individual's cells having been occupied by non-OXPHOS mitochondria at the approach of old age. One of the consequences of having non-OXPHOS mitochondria is the depletion of the supply of NAD+, an essential carrier molecule in OXPHOS. This is a consequence of the absence of a properly functioning OXPHOS process returning NAD+ into circulation, in turn, preventing a toxic excess of NADH from accumulating. Nevertheless, in this situation, another means to recycle NADH into NAD+ can be employed. Since NADH is primarily NAD plus an electron, the cell simply exports the unwanted electrons by using components on the cell membrane, known as the plasma membrane redox system (PMRS), recycling it into NAD+. The electrons accumulating on the membrane then combine with oxygen molecules, creating reactive oxygen species, which add to the already generated accruement, causing further oxidative damage as part of a 'vicious circle'.

Another way in which decreased OXPHOS function could increase ROS production further is via the ensued slowing of the electron transport chain. Studies have found that when the flow of electrons is slow, the tendency of electrons to accumulate in the respiratory chain increases, in turn, causing more electrons to escape from the "leaky" ETC. Thus, their capacity to form ROS is increased, ultimately leading to a rise in the number of ROS (Kushnareva et al., 2002; Trifunovic and Larsson, 2008). This notion is supported by the fact that selectively decreasing complex IV of the electron transport chain, increases the production of ROS (Atamna et al., 2001), thus slowing the rate of electron flow. Not only that, administration of sodium azide, an inhibitor of complex IV, did not just increase the production of ROS from the mitochondrial electron transport chain, but also impaired learning and memory in rats (Callaway et al., 2002; Cassarino and Bennett, 1999), suggesting a causal relationship between ROS production – as a result of ETC dysfunction – and AD.

The mitochondrial theory of aging is further supported by a large number of studies supplying evidence that the aging human brain exhibits an increase in oxidative damage to proteins (Oliver et al.,

1987; Manczak et al., 2004), in particular, mtDNA, which correlates with age-associated mitochondrial dysfunction (Cottrell and Turnbull, 2000, Richter et al., 1988). Although the observed mitochondrial DNA mutations can be a direct consequence of sporadic errors during mitochondrial DNA replication (Kujoth et al., 2007), the large accumulation of mutations observed at old age is said to occur due to mtDNA replication errors resulting from oxidative damage (Hiona and Leeuwenburgh, 2008). The observed mtDNA changes include oxidative damage to DNA bases, point mutations and large scale deletions or duplications (Wanagat et al., 2001; Wang et al., 2001; Kraytsberg et al., 2006). A number of factors appear to be responsible for the susceptibility of the mitochondrial genome to oxidative damage, including, mtDNA's close proximity to the ETC – increasing its exposure to reactants – the lack of protective histones and introns, and the compactness of its genetic information, enhancing the likelihood that damage to any point in the genome will occur in a gene (Hiona and Leeuwenburgh, 2008).

The question now, however, is whether mitochondrial dysfunction and oxidative stress in old age are actually responsible for Alzheimer's disease. Oxidative stress has been found to occur in mitochondria of the brain, platelets and fibroblasts from AD patients (Beal, 2005; Reddy, 2007). Oxidative stress was also reported in the brains of AD patients (Gibson et al., 1998; Parker et al., 1990; Maurer et al., 2000; Smith et al., 1996; Hirai et al., 2001) and in those of AD transgenic mice (Caspersen et al., 2005; Lustbader et al., 2005, Anandatheerthavarada et al., 2003; Li et al., 2004, Smith et al., 1998). Furthermore, double-labelling immunofluorescence analysis conducted by Manczak and colleagues (2004) revealed that oxidative damage, marked by 8-hydroxydeoxyguanosine (8-OHG), was increased mainly in the AD neurons showing increased cytochrome oxidase, suggesting that mitochondrial dysfunction and oxidative damage are linked in AD brains. Thus, the difference between normal aging and AD could lie in the threshold of cell impairment in response to oxidative damage and abnormal mitochondria. However, not all neurons with overexpressed COX were positive for 8-OHG immunoreactivity. Although this could mean that neurons undergo oxidative damage at different stages in AD patients, it could also insinuate that oxidative damage is simply not responsible for all cases of altered mitochondrial function and, in turn, Alzheimer's disease.

In line with this, many mouse models with impaired mitochondrial function display only minor or no oxidative stress (Trifunovic et al., 2005; Wang et al., 2001; Kujoth et al., 2005). One particular mouse model, known as the 'mutator mice', has been said to "show no causal relation between the accumulation of mtDNA mutations and elevated ROS production and therefore argues against any direct role of oxidative stress in the aging process" (Trifunovic et al., 2005). The 'mutator mice' express a proofreading-deficient mitochondrial DNA polymerase (POLG), which causes them to accumulate mtDNA mutations in an age-dependent manner and develop many features of premature aging, specifically cardiomyopathy, kyphosis, reduced subcutaneous fat, osteoporosis, anemia, reduced fertility and a significant decrease in lifespan to one year of age (Trifunovic et al., 2004). Although embryonic fibroblasts derived from mutator mice displayed defects in OXPHOS function, at the same time, however, those cells and tissues did not exhibit elevated ROS production or oxidative damage (Fukui et al., 2008). Even though one can argue that specific mutations in OXPHOS components and related functions are required for (increased) ROS production, the random nature of mutations in these mice would be expected to give rise to such defects. Thus, the lack of oxidative stress in this model of aging raises the question whether age-dependent accumulation of mtDNA mutations and consequent decreased mitochondrial function are actually responsible for increased oxidative damage, as was proposed by the mitochondrial theory of aging. Nevertheless, the mouse model should be interpreted with caution, as the 'mutator mice' do not encapsulate the complete aging phenotype. Reports of any immediate central nervous system (CNS) phenotypes were unclear and it stimply cannot be ruled out that the mice died of sickness (e.g. cardiomyopathy) instead of rapid aging (Melov, 2004). Also, although it was reported that no dramatic intracellular increase in ROS production was measured, unless the measurements occurred for a long period of time *in vivo*, it simply cannot be determined whether the steady-state levels of reactive oxygen species were just a product of an effective, fully functional, antioxidant system.

Indeed, another important factor determining the steady-state levels of the brain's oxidative damaged molecules is the antioxidant capacity of the ROS defense mechanisms. Increased oxidative damage and impaired antioxidant defenses have been shown to be prominent and early features of AD brains (Smith et al., 1997; Straface et al., 2005). Marcus and colleagues (1998) demonstrated that the activities of the antioxidant enzymes copper/zinc superoxide dismutase (Cu/ZnSOD) and catalase are significantly decreased in the frontal and temporal cortex of AD patients. Not only that, antioxidant enzyme activity was shown to be spatially correlated with markers of lipid peroxidation (i.e. oxidative damage) and the brain areas particularly affected by neuronal loss in AD (Takeda et al., 2000; Zhu et al., 2003). However, increased antioxidant activity in AD brains in response to increased free radical generation has also been reported (Lovell et al., 1995; Lu et al., 2004; Moreira et al., 2009). Thus, one explanation for the studies showing a lack of correlation between impaired mitochondrial function and oxidative stress is that in some AD cases, antioxidant defenses are able to counteract oxidative stress damage, whilst in others they cannot. However, this would automatically mean that in the AD cases with no oxidative stress, other impaired mechanisms must be causing AD.

5. The relationship between amyloid-β, mitochondrial dysfunction and AD pathology

As was discussed in the first chapter, soluble Aβ instead of insoluble amyloid plaques was shown to impair synaptic function. The second chapter described specific brain regions as experiencing decreased glucose metabolism in early AD. These brain regions of impaired glucose utilization corresponded with the ones showing reduced expression of genes encoding components of the electron transport chain in mitochondria. Moreover, as glucose is utilized by mitochondria, the possible role of mitochondrial dysfunction in aging and AD was therefore discussed in chapter 2. However, as the relationship between age-related oxidative stress and mitochondrial dysfunction is still, to some extent, dubious, another culprit may provide the missing key. As soluble Aβ was shown to be inextricably implicated in AD, it is not surprising that recent studies have demonstrated a strong link between the mitochondrial and amyloid hypotheses.

Various cell models have shown decreased mitochondrial function to be related to the mitochondrial accumulation of full-length amyloid precursor protein and Aβ (Anandatheerthavarada et al., 2003; Devi et al., 2006; Keil et al., 2004; Park et al., 2006). In human cortical neuronal cells (HCN cells), decreased mitochondrial membrane potential, ATP levels, and mitochondrial cytochrome oxidase activity were shown to be associated with the accumulation of amyloid precursor protein (APP695) in mitochondria (Anandatheerthavarada et al., 2003). However, accumulation of APP lacking the acidic domain caused no reduction in COX activity or impaired energy metabolism. This suggests that the incomplete mitochondrial translocation of APP mediated by the acidic domain is required for mitochondrial dysfunction to occur (Anandatheerthavarada et al., 2003). Furthermore, isolated rat brain mitochondria incubated with Aβ experienced lowered state 3 and 4 of respiration due to inhibitory binding to COX, but the researchers could not rule out a contribution by inhibition of other enzymes such as α-KGDHC and PDHC for substrates upstream of these enzymes (Casley et al., 2002). Not only that, COX activity was reduced in transgenic mice expressing mutant APP (Tg2576) mice compared to age-matched wild-type littermates (Schmidt et al. 2007). Altogether, this suggests that mutant amyloid precursor protein and soluble Aβ are involved in impaired mitochondrial function in AD development and progression.

In fact, Aβ was found to be temporally related to mitochondrial dysfunction and aging. Caspersen and colleagues (2005) found a significant 12-month low in oxygen consumption and activities of respiratory enzymes succinate-cytochrome *c* reductase (complex III) and COX in transgenic mutant APP (Tg mAPP) mice to coincide with a significant peak of accumulated Aβ42 at 12 months. Since wild type littermates experienced no changes in oxygen consumption or enzyme activity, the results indicate that accumulation of Aβ within mitochondria correlates temporally with changes in mitochondrial function. Not only that, as the onset of Aβ was found to be at levels just above base-line at 4 months of age, reaching a peak at 12 months, it suggests that soluble Aβ production follows an age-related trend. This notion is supported by time-course analyses of Aβ in transgenic AD mouse lines and post-mortem brain studies of elderly individuals with mild cognitive impairment and AD patients (Gouras et al., 2000), which showed an age-related increase in Aβ production and deposits (Manczak et al., 2006; Oddo et al., 2003). Thus, aging most likely has a significant role in the production and deposition of Aβ in brains of AD patients and transgenic AD mice. In light of the previous chapter, it should therefore not be surprising that mitochondrial dysfunction has also been found to be intricately linked to the aging process.

One major, common denominator in the majority of studies discussed so far, is a reduction in cytochrome oxidase activity. Although the amyloid studies suggest that Aβ is responsible for this reduction, this relation is most likely more causal than direct. Thus, several groups have tried to elucidate the molecular mechanisms underlying this COX defect. To begin with, there is no question about the fact that amyloid β-peptide can exert its effects intracellulary, as studies have shown it to be imported into mitochondria and locate on the structures inside. Hansson Petersen and colleagues

(2008) showed that the transportation into mitochondria occurs via the translocase of the outer mitochondrial membrane (TOMM) machinery. However, this means that Aβ is one of more than 1500 nuclear encoded proteins reported to be associated with and transported into mammalian mitochondria via import channels (Taylor et al., 2003; Gabaldon and Huynen, 2004), so this data by itself does not say much. Nevertheless, subfractionation studies demonstrated that Aβ associates with the inner membrane fraction, the structure in which the electron transport chain is located, and immunoelectron microscopy revealed localization of Aβ to mitochondrial cristae. This distribution pattern of Aβ in mitochondria correlated with the pattern in "human cortical brain biopsies obtained from living subjects with normal pressure hydrocephalus" (Hansson Petersen et al., 2008), and can thus be said to apply to *in vivo* situations as well.

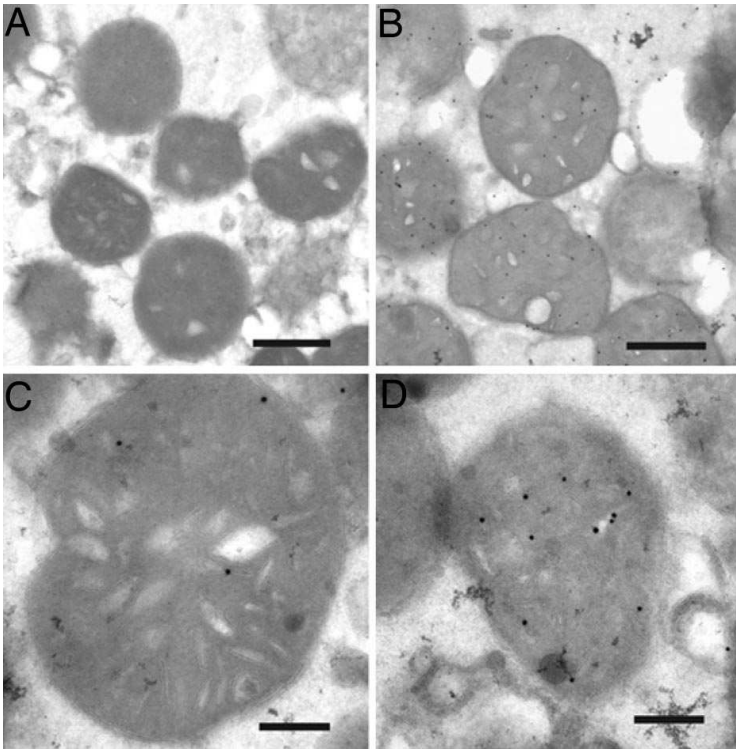

Figure 2. **Immunoelectron microscopy image showing the cellular location of Aβ1-42**. Gold particle labeling of Aβ1-42 shows that the peptide was imported into mitochondria and associated to the inner mitochondrial membranes. Quantification of the distribution of Aβ1-42 inside mitochondria after import showed that ~75% of the gold particles were detected in cristae, 18% in matrix, and 7% associated with the outer membrane. In control samples treated the same way, no A Aβ1-42 was added during import. (Image taken from Hansson Petersen et al., 2008).

Secondly, studies have shown Aβ to also exert its effects by specifically blocking the entry of nuclear-encoded proteins into the mitochondria, resulting in increased oxidative stress. This, of course, separates it functionally from the other imported mitochondrial proteins. Data from *in vitro* studies by Sirk and colleagues (2007) demonstrated that sub-lethal concentrations of Aβ(25-35) or Aβ(1-42) blocks the entry of nuclear-encoded proteins into mitochondria when PC12 cells are exposed to this peptide. This blockage was found to decrease mitochondrial membrane potential, increase ROS production and oxygen glucose deprivation, and alter the morphology of mitochondria. Devi and colleagues (2006) found that APP accumulates across mitochondrial import channels in a similar fashion, but not only did the extent of accumulation correlate with the severity of AD, it specifically inhibited entry of nuclear-encoded cytochrome subunits IV and Vb proteins. This, in turn, was associated with decreased cytochrome oxidase activity and increased levels of hydrogen peroxide. The increased ROS experienced as a result of Aβ pore blockage was suggested to be due to decreased COX activity increasing electron leak from upstream components of the respiratory chain, such as ubiquinone and cytochrome b566 in complex III.

Thirdly, Aβ has been found to interact with Aβ-binding alcohol dehydrogenase (ABAD) in the mitochondrial matrix. Immunoprecipitation of AD brain showed that ABAD and Aβ interact in homogenates and mitochondrial extracts (Lustbader et al., 2004). Moreover, blocking this interaction with a "decoy peptide", i.e., an ABAD peptide with a human binding region, which binds competitively to the original peptide, suppressed Aβ-induced apoptosis and ROS production in neurons (Wu et al., 2010). On the contrary, overexpression of ABAD in transgenic mutant APP mice exacerbated mitochondrial abnormalities and oxidative stress in neurons (Lustbader et al., 2004). A study by Takuma et al. (2005) showed that neurons cultured from transgenic mice overexpressing a mutant form of APP and ABAD (Tg mAPP/ABAD) display "spontaneous generation of ROS, decreased ATP production, COX activity and glucose utilization, subsequent release of cytochrome *c* from mitochondria with subsequent induction of caspase-3-like activity followed by DNA fragmentation and loss of cell viability" (Takuma et al., 2005). Interestingly, these changes closely correspond with hippocampal and neurophysiological impairment, learning and memory deficiency, in particular, basal synaptic transmission (BST) in the hippocampal callicebus cornus ammonis 1 (CA1) stratum radiatum, as well as long-term potentiation impairment. These results therefore provide support for the notion that ABAD serves as a "cellular co-factor" (Chen and Yan, 2007) promoting and amplifying Aβ-induced mitochondrial impairment, leading to neuronal and synaptic dysfunction in an AD-like manner.

Fourthly, metabolic impairments and oxidative stress have been shown to occur before plaque formation (Mouche et al., 2007), which reiterates findings from AD brain tissue studies and suggests that mitochondrial impairment is a significant early factor in the development of AD pathology. Oxidative damage was also found to occur before Aβ deposition in transgenic APP mice (Droge, 2002), with mitochondrial metabolism and apoptosis-related genes upregulated even earlier in oxidatively damaged neurons (Linnane et al., 2007). This is in line with recent studies demonstrating that oxidative stress *in vitro* induces increased expression of BACE1 and PS1, thereby enhancing Aβ production (Tong et al., 2005; Tamagno et al., 2005; 2008).

However, *in vitro* data from toxicity studies have also indicated that cells exposed to high concentrations of amyloid-β (Aβ) release large amounts of hydrogen peroxide, i.e. ROS (Behl et al., 1992; 1994) and that antioxidants offset Aβ-induced hydrogen peroxide toxicity (Behl et al., 1992). Thus, as Aβ is evidently a source of oxidative stress, and oxidative stress is clearly involved in Aβ accumulation, these two influences appear to participate in a vicious cycle, resulting in JNK/c-jun activation (Tong et al., 2005; Tamagno et al., 2005) and enhanced levels of BACE1 and γ-secretase, which further increases Aβ production.

Su and colleagues (2008) have demonstrated that oxidative damage is reduced by the formation of neurofibrillary tangles (Nunomura et al., 2001). As neurons with neurofibrillary tangles can survive for years, a fact that is consistent with data from mouse models demonstrating that NFTs are not implicated in neuronal death (Su et al., 2008; Andorfer et al., 2005; Santacruz et al., 2005), it is possible that the formation of neurofibrillary pathology is "a further neuronal adaptation to chronic oxidative stress" (Su et al., 2008; Lee et al., 2005). In line with this, it was recently shown that intracellular accumulated β-amyloid precedes both NFTs and synaptic dysfunction in transgenic mutant Aβ, presenilin, and tau mice. Therefore, in a chronic oxidative stress situation, when antioxidant enzymes are unable to offset the toxic culprits, as appears to be the case in Alzheimer's disease, neuronal cells may resort to other adaptations, such as the phosphorylation of tau protein via the activation of c-Jun N-terminal kinases, which play a role in one of the stress-activated protein kinase (SAPK) pathways, to form neurofibrillary tangles, as a means to provide alternative antioxidant activity (Nunomura, 2001).

However, one aspect of Aβ that has not yet been touched upon is that the peptide may, in fact, serve as a response to neuronal injury, rather than a mediator of such an injury (Lee et al., 2004). Aβ and APP have been shown to occur after oxidative stress and, rather than increasing stress, actually appear to *decrease* oxidative stress *in vivo* (Nunomura, 2000; 2001) by acting as an antioxidant (Curtain, 2001). Consistent with this response, Aβ has been detected in the human brain several days after traumatic

brain injury (Gentleman et al., 1993), which suggests APP may act as an "acute phase reactant" (Lee et al., 2004), as it is upregulated in neurons, astrocytes and microglial cells in response to inflammation and various other cellular stresses, such as axonal injury (Blumbergs et al., 1995; Gentleman et al., 1993), loss of innervation (Wallace et al., 1993), excitotoxic stress (Panegyres, 1998; Topper et al., 1995), heat shock (Ciallella et al., 1994), oxidative stress (Frederikse et al., 1996; Yan et al., 1994), aging (Higgins et al., 1990; Nordstedt et al., 1991, van Gool et al., 1994) and inflammatory processes (Brugg et al., 1995; Buxbaum et al., 1992; Buxbaum et al., 1998). Furthermore, when the energy supply is depleted in conditions such as ischemia, hypoglycemia and traumatic brain injury, known to put neurons under oxidative stress, APP protein and mRNA have been found to be upregulated in animal models and culture systems (Hall et al., 1995; Jendroska et al., 1995, Murakami et al., 1998; Shi et al., 1997). In line with this, inhibition of mitochondrial metabolism switches the processing of APP to "generate amyloidogenic derivatives" (Swerdlow et al., 2010; Frederikse et al., 1996; Gabuzda et al., 1994; Mattson and Pedersen, 1998). However, it must not be forgotten that Aβ has also been shown to induce oxidative stress. This seems to suggest that in situations of acute, short-term stress, Aβ may function to decrease the consequent neural injury/stress perhaps by stimulating the antioxidant response system via oxidative stress, but when the increase in Aβ due to old age and mitochondrial dysfunction is paired with faltering antioxidant capacity as a result of age, the Aβ-induced oxidative stress will remain unchecked, thus giving Aβ 'oxidative properties'.

In line with this, Swerdlow and Khan (2009) suggest that in autosomal dominant Alzheimer's disease, the primary cause of mitochondrial dysfunction is the overproduction of Aβ whilst in sporadic Alzheimer's disease the main culprit is the decline of mitochondrial function beyond an age-related threshold. Aβ, one of the downstream products of the mitochondrial impairment, will thereafter act as part of a positive feedback loop that exacerbates mitochondrial decline (*see figure 3 for a visual overview of the main elements discussed so far*) (Swerdlow and Khan, 2009). In fact, Swerdlow and Khan (2009) proposed "that cells may have developed this loop to shut down perturbed mitochondria, or else help cells in the transition from aerobic to anaerobic metabolism." In any case, they believe Aβ did not evolve "solely as a toxic time bomb".

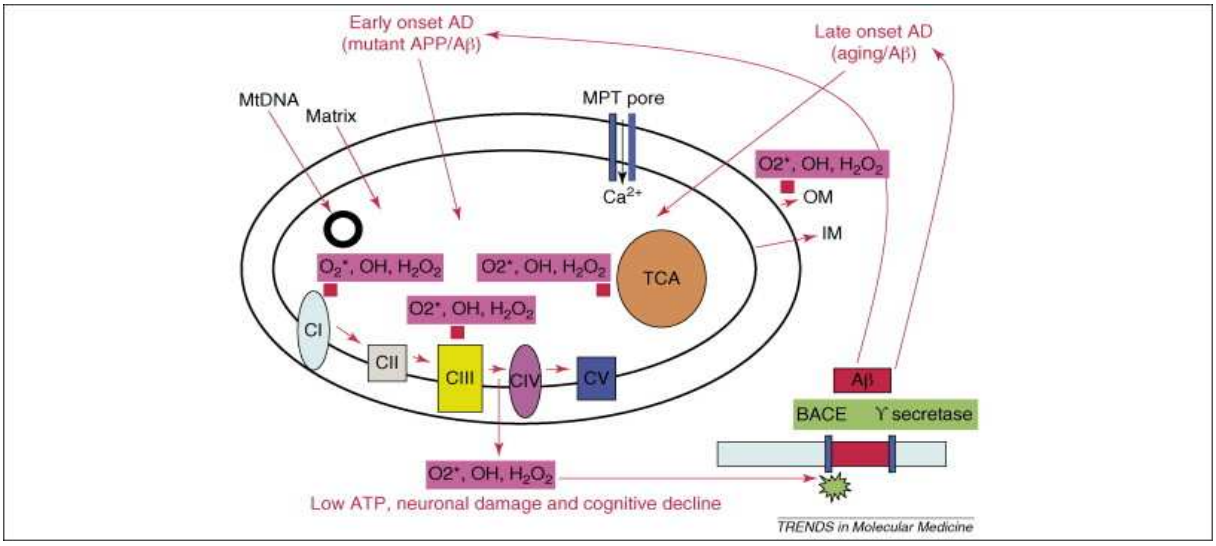

Figure 3. **A visual overview of some of the key elements discussed so far**.
In early-onset Alzheimer's disease, mutant APP and soluble Aβ are hypothesized to localize to synaptic mitochondria, leading to the production of free radicals ($O_2\cdot$, H_2O_2, OH). Subsequently, the free radicals may decrease cytochrome oxidase activity and inhibit cellular ATP. In late-onset Alzheimer's disease, on the other hand, the age-related accruement of ROS may activate BACE1 and facilitate the cleavage of the Ab. The generated Aβ may thereafter translocate to mitochondria and induce further production of free radicals ($O_2\cdot$, H_2O_2, OH), leading to the disruption of the ETC, a decrease in cytochrome oxidase activity and the inhibition of ATP. This viscious cycle may ultimately lead to neuronal damage, the degeneration of neurons and decline of cognitive functions, known as Alzheimer's disease. (Image taken Reddy & Beal, 2008).

6. ‘Use it or lose it’: engaging in physical and mental activities prevents Alzheimer’s disease

In aging humans, the cortex and hippocampus atrophy (Golomb et al., 1996) and memory function declines (Gazzaley et al., 2008). Recently, society has placed particular emphasis on the benefits of physical exercise and sufficient mental stimulation, claiming that the deleterious consequences of aging can be attenuated by exercise and stimulation from the environment. Evidence demonstrating enhanced cognitive functioning in older adults and mice as a result of aerobic physical exercise and environmental stimulation has indeed been overwhelming. In humans, environmental stimulation is achieved through an engaged lifestyle, potentially involving a wide range of activities. The equivalent of an engaged lifestyle in animals is known as ‘environmental enrichment’ and involves increased stimulation and activity opportunities in their environment through toys and running wheels.

Evidence supporting the beneficial effects from physical activity and an engaged lifestyle highly tallies with the ‘use it or lose it’ hypothesis. Physical exercise enhances glucose uptake (Fox et al., 1988; Lopez-Lopez et al., 2004) as neurons are required for the planning and execution of movement. In fact, a crucial region affected by Alzheimer’s disease, the hippocampus, was shown to experience increased neuronal activity in response to exercise (Czurkó et al., 1999). Needless to say, the variety of activities comprising an engaged lifestyle require its share of cognitive effort as well. In fact, intuitively it could be said that the brain regions stimulated during engaging lifestyle activities might be more relevant in light of the cognitive functions affected by AD than those activated by physical exercise. Nevertheless, the effects of exercise and an engaging lifestyle can therefore be deemed to be highly relevant to the ‘use it or lose it’ hypothesis. This chapter will discuss the results of studies that investigated the effects of aerobic exercise and an engaged lifestyle separately, as well as describe the ways in which these benefits are manifested.

Part 1 – The cognitive and physiological effects of physical exercise on the brain

i. The effects of exercise on the human brain

On the whole, the notion that exercise prevents cognitive deterioration is supported by longitudinal studies of markers of cognitive decline and prevalence of dementia, as well as intervention studies in human and animal populations. “Physically fit aged individuals, identified by self-report of activity level, performed superiorly on measures such as working memory, vocabulary, reaction time and reasoning compared to their sedentary equivalents” (Van Praag, 2009; Yaffe et al., 2001). In intervention studies, 60 to 85 year old healthy sedentary adults take part in an exercise regime several times a week over the course of several months to several years. Their cognitive abilities and fitness were assessed before and after the intervention. Although the studies differed in duration, intensity and type of exercise, overall physical activity was shown to improve cognitive function (Hillman et al., 2008, Kramer et al., 1999). On the whole, exercise-intervention work so far has demonstrated relatively general cognitive benefits of aerobic exercise, but cognitive tasks requiring executive functioning, working memory, and attentional control have been shown to benefit the most (Hertzog, 2009). Furthermore, these benefits were mirrored by neurophysiological measures such as electroencephalogram, event related potential (ERP) and functional Magnetic Resonance Imaging studies (fMRI) (Hillman et al., 2008). ERP latency and amplitude were shown to be decreased and increased, respectively, in physically fit individuals, suggesting neuronal conduction and cortical activation had improved (Hillman et al., 2004). MRI studies, on the other hand, showed that “prefrontal and temporal gray matter volume was increased in active elderly subjects compared to sedentary controls” (Colcombe et al., 2003).

A question particularly relevant with regard to the cognitive-enrichment hypothesis is whether individual differences in physical activity at earlier points in life span influence cognitive decline amongst the elderly. Although a positive correlation between physical activities at ages 15–25 and information processing speed in older men (62–85 years of age) was reported (Dik et al., 2003), the influence of past activity participation does not appear to trump the benefits of current activity levels. In fact, Wilson and colleagues (2005) found that the association between current activity and current cognitive ability was stronger than those with past activity (Calero-García et al., 2007). Moreover, they found the relationships between past activity and current cognition to be reduced after controlling for current cognitive activity, but controlling for past engagement did not affect the relationship between current activity and current cognitive ability. Similarly, Richards et al. (2003) found physical activity at 43 years of age to be a better predictor of verbal memory change from 43 to 53 years of age than the amount of physical activity participated in at age 36. Furthermore, they found little evidence of protection against memory decline for those who stopped exercising after age 36, showing that the benefit of past activity was lost if activity was not maintained. On the other hand, those who started physical activity after age 36 were protected, and being physically engaged at both occasions had an additive effect, as the cognitive benefits of past activity were enhanced by more recent activity. Thus, although aerobic exercise at a young age might increase the resilience of the brain later in life due to an increase in "cognitive reserve" (Stern, 2006), additional physical activity is still needed at an older age, possibly to combat the age-related brain alterations that predispose individuals to AD.

ii. The effects of exercise on the rodent brain

Studies on adult rodents have shown exercise to be equally beneficial. To begin with, both voluntary and forced exercise improved spatial memory in Morris water maze, Y-maze, T-maze and radial arm maze tests (Van Praag, 2008). Running was also found to enhance "performance in hippocampus-dependent tasks involving limited movement, such as contextual fear conditioning, passive avoidance learning and novel object recognition" (Liu et al., 2008; O'Callaghan et al., 2007). Moreover, "non-hippocampal dependent, anxiety-related behaviour, such as performance in the elevated plus maze (Greenwood et al., 2003), benefits from voluntary and forced exercise." Even though, at this point, it is unclear whether voluntary and forced exercise have equivalent effects, the degree of behavioural and cellular effects have been found to differ even when the activity parameters in both paradigms are nearly identical (Leasure and Jones, 2008). Similar results were obtained in studies using transgenic mouse models for AD, as not only did long-term exercise, started 5 months before disease onset, improve water-maze learning, but short-term running (3 weeks), started after disease onset, also enhanced both working and reference memory in aged AD mutant mice (Nichol et al., 2007). Clearly, exercise has a positive influence on cognition in both transgenic mouse models of dementia and normal rodents, regardless of whether it was instigated later in life or after AD onset**.**

The cellular and physiological mechanisms underlying the improved cognitive function as a result of exercise vary widely. To begin with, exercise causes enhanced synaptic plasticity in rodents that run due to neural structural changes. "Hippocampal tissue slices experienced enhanced long-term potentiation in the dentate gyrus of running versus sedentary mice" (Van Praag et al., 1999) and *in vivo* experiments showed similar changes in rats that were housed with a running wheel (Farmer et al., 2004) or given forced treadmill exercise (O'Callaghan et al., 2007). Enhancements in LTP are partly mediated by changes in fine cell morphology. Exercise particularly changes the properties of dendritic spines, the actin-rich protrusions on dendrites forming excitatory synapses (Van Praag, 2009). Changes in spine size and quantity were shown to be related to LTP induction and are thought to support alterations in synaptic strength (Van Praag, 2009). Recently, a study demonstrated that running augmented spine density in the dentate gyrus, area CA1 and entorhinal cortex layer III (Stranahan et al., 2007).

Enhanced long-term potentiation in the rodent hippocampus is partly the result of another exercise-induced mechanism, namely neurogenesis. Neurons of the adult mammalian brain are replenished in

the olfactory bulb and dentate gyrus of the hippocampus throughout life. This process has been increasingly shown to be implicated in learning and memory, as new cells were preferentially activated during learning tasks (Kee, 2007) and enhanced neurogenesis has been associated with improved cognition (Van Praag, 2009). Studies have shown that wheel running increases the production and survival of new neurons in the dentate gyrus of the rodent hippocampus 3-4-fold (Van Praag, 2008), with cell genesis peaking at three days. Although the new cells comprise a tiny fraction of the granule cell layer, these neurons have been shown to cause a transient increase in LTP amplitude and a decreased induction threshold (Schmidt-Hieber et al., 2004; Ge et al., 2007), and might therefore explain the overall enhanced LTP observed in the hippocampus of rodents. Moreover, as synaptic plasticity has been found to be impaired in AD brains, it would only makes sense if some of the benefits of physical exercise are realized by the addition of new, functional neurons to the synaptic dysfunctional hippocampus.

The exercise-induced changes in neurogenesis can occur throughout life in rodents. “In mice that exercised continuously from young to middle age, the normal age-related decline in cell genesis was significantly less than in their sedentary counterparts” (Van Praag, 2009; Kronenberg et al., 2006). Moreover, in mice that started wheel running in middle age (Wu et al., 2008) or old age (Van Praag et al., 2005), the number of new neurons was significantly increased. Furthermore, it was reported that exercise can reverse pregnancy- (Rolls et al., 2008) and radiation-treatment-related (Naylor et al., 2008) decline in hippocampal neurogenesis. In specific transgenic mouse models for neurological disease, however, the enhanced neurogenesis experienced as a result of physical activity has been shown to be equivocal (Van Praag, 2008). Transgenic mice that express human presenilin-1 variants linked to early-onset familial AD do not show exercise induced neurogenesis due to impaired proliferation and neuronal differentiation of hippocampal neural precursor cells (NPCs) via non-cell autonomous mechanism(s) (Choi et al., 2008). Therefore, the neurogenic and cognitive effects of physical activity may not be beneficial to all forms of AD.

Apart from neurogenesis, physical activity has been shown to stimulate angiogenesis (Van Praag et al., 1999; Pereirra et al., 2007) by increasing the proliferation of brain endothelial cells throughout the brain (Cotman et al., 2007). The growth factors insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF) were shown to have significant roles “in the angiogenic effects of exercise on the brain, as running enhanced hippocampal IGF gene expression (Carro et al., 2000) and increased serum levels of both IGF (Carro et al., 2000) and VEGF (Fabel et al., 2003)”. Furthermore, Lopez-Lopez et al. (2004) showed that exercise-induced angiogenesis may be specifically stimulated to provide larger cerebral blood volume (CBV) in response to the increased metabolism in various brain regions, including the hippocampus. As cerebral perfusion is a noted measure for neural activity (Iadecola, 2004), and regional hypoperfusion, prominently associated with a reduction in cerebral metabolism (Attwell and Iadecola, 2002; Wolf et al., 2001), is a hallmark of AD and contributes to cognitive decline (Miklossy, 2003; Swaab, 1991, 2004), it seems to be logical for exercise to have such a positive effect on cognitive functioning.

However, the beneficial effects of exercise on cerebral perfusion seem to be intricately tied to the patient’s cardiac condition. Koike and co-workers (2004) observed that patients with valvular heart disease, and hence decreased cardiac output, experienced declined cerebral oxyhemoglobin levels during increasing physical effort on a cycle ergometer, i.e. an instrument that measures the amount of work done. Thus, during exercise, the blood flow to muscle cells in these patients occurs at the expense of blood flow to other organs such as the cerebral cortex. Others have confirmed that “exercise combined with limited cardiac output and a large muscle mass affects the regional increase in cerebral perfusion” (Eggermont et al., 2006; Ide et al., 1998). Furthermore, studies have shown that cardiovascular disorders like hypertension increase the risk of AD (Skoog and Gustafson, 2003; De la Torre, 2002). In fact, high systolic blood pressure enhances the risk for hippocampal atrophy and therefore the risk for AD (Launer et al., 2000). Thus, not only do the benefits of exercise appear to bypass individuals with altered prenisilin expression like in familial forms of AD, but possibly those with particular cardiovascular problems as well.

Another effect exercise may have in individuals that could prove to be beneficial, is an increase in mitochondrial activity and number. At this point, the majority of studies have focused on skeletal muscle, one of which demonstrated a 53 percent increase in mitochondrial number in the muscles of previously sedentary individuals with an average age of 67, who had been put on a program of 30-40 minutes of aerobic exercise 4 to 6 times a week (Menshikova et al., 2006). This increase occurred after only 12 weeks and was concomitant with a 62 percent increase in the amount of energy produced. As energy metabolism in neurons has also been shown to be enhanced in response to exercise, it would only be natural to expect an increase in the number of mitochondria in neurons as well. Indeed, Dietrich and colleagues (2008) showed similar mechanisms in hippocampal neurons, where exercise enhanced mitochondrial respiration and number, which in turn was thought to contribute to an overall increase in ATP production (Diano et al., 2003; Andrews et al., 2005a). Thus, although mitochondrial replication may create a reserve of dysfunctional mitochondria, it could also very well increase the number of highly functional mitochondria, which could offset the effects of the poorly working ones, thus, delaying the onset of Alzheimer's disease.

Some of the changes that occur as a result of exercise have been found to be closely tied to the mitochondrial theory of aging. With regards to this theory, one would expect that an increased metabolic rate and associated oxygen consumption would increase the amount of ROS production as a result of aerobic exercise. Although this has indeed been found to be the case (Packer et al., 2008; Leeuwenburgh et al., 1994; Sastre et al., 1992), recently free radical production was shown to actually *decrease* in response to a prolonged period of moderate physical activity (Trifunovic and Larsson, 2007; Sanz and Stefanatos, 2008). This was related to the fact that mitochondrial ROS generation was found to be high during resting metabolic rate (RMR) as the rate of oxygen consumption (VO_2) is low in this state. Thus, when mitochondrial oxygen consumption acutely increases, as during active exercise, the local depletion of O_2 occuring within mitochondria, consequently limits ROS production due to lack of one of its substrates (Hoffman et al., 2007). With this in mind, one would therefore expect a causal relation between oxidative stress markers and RMR instead of total energy expenditure (Frisard et al., 2006), as confirmed by Loft and colleagues (Loft et al., 1994).

All this, of course, is rather contrasting to the mitochondrial theory of aging, which has gone so far as to show that increased physical activity in houseflies enhances metabolic rate and ROS production, consequently decreasing their lifespan. The results of these types of studies, however, are questionable, as they neglected to separate resting metabolic rate from thermogenesis and physical activity, which are all components of total daily energy expenditure (TDEE) (Ravussin and Swinburn, 1993). Moreover, the notion that an increased metabolic rate and the consequent ROS production (due to physical activity) decreases life-span can be said to be flawed simply by looking at the bat and the shrew whose RMRs are equally high, yet the bat can live 32 years longer than the shrew. Unfortunately, the relationship between energy expenditure and oxidative stress still needs to be investigated thoroughly, as well as the association with RMR in humans and the effects of age on this relationship (Loft et al., 1994).

However, what has to be kept in mind is that the studies are still largely divided on the matter of ROS production during exercise. Also, the majority of the studies claiming to have identified significant decreases/increases in free radical content in the nervous system of rodents are not always based on direct measures of ROS production, but levels of oxidative damage that were either decreased or simply did not increase. Jolitha et al. (2006) found decreased lipid and protein oxidation in the cerebral cortex, hippocampus and cerebellum of adult rats that swam 40 days, 30 minutes a day. Furthermore, levels of oxidized glutathione (GSSG) decreased whilst reduced glutathione (GSH) levels were unchanged in the cerebral cortex and striatum of adult rats that ran 7.5 weeks on a treadmill (Somani et al, 1995). Young rats with unlimited access to the running wheel for a course of 14 or 28 days had reduced brain lipoperoxidation levels (Suzuki et al, 1983). Furthermore, neither Ogonovszky and colleagues (2005) nor Radák et al. (2006) found decreased oxidative damage to DNA and lipids in the brain of old rats that swam strenuously. In fact, both studies found improved memory after the long period of training. However, the point to consider here is that for the majority of studies it is not completely clear whether oxidative damage decreased/failed to increase because of decreased ROS

production or because of alterations in other related mechanisms, such as the upregulation of antioxidant defense systems.

Several studies have found antioxidant mechanisms to be upregulated after exercise, which some studies owe to an adaptive response to *increased* ROS production during physical activity (Murphy et al., 2003; Goto and Radak, 2007). Regardless of this, one of these studies found the glutathione system to be upregulated (Leeuwenburgh et al., 1994), whereas another found the adaptive response to encompass "increased antioxidant defenses, reduced basal production of oxidants, and reduction of radical leak during oxidative phosphorylation" (Leeuwenburgh and Heinecke, 2001). In fact, the diminished leak from the ETC could be due to the induction of uncoupling proteins (UCPs) (Murphy et al., 2003), which play a role in decreasing the release of electrons from the electron transport chain, in so doing, attenuating the production and build up of ROS (Brand et al., 2000). All in all, it is very much possible that the reduction in oxidative stress due to the adaptations of the antioxidant system as a result of exercise, are partly responsible for the cognitive benefits experienced by exercising.

To sum up, although the literature is still divided on the production of ROS during exercise, regardless of the true data, the benefits from physical exercise, as described earlier, are so abundant, that they most likely would moderate the negative consequences of increased ROS production anyhow.

Part 2 – The cognitive and physiological effects of an engaged lifestyle on the brain

iii. The effects of an engaged lifestyle on cognitive function in humans

The second part of this chapter will focus on the benefits attained in cognition as a result of an engaged lifestyle in old and early age. The 'use it or lose it' hypothesis of cognitive aging predicts "that engagement in physical, social, and intellectual activities in older adulthood prevents the deterioration of cognitive abilities by 'exercising' them through their application in various environments" (Salthouse, 1991; Bielak, 2009). Individuals who partake in various activities are predicted to perform better on cognitive tests, experience less cognitive decline over time, and possibly even have a decreased risk of developing Alzheimer's disease compared to sedentary individuals (Fratiglioni, 2004; Small et al., 2007).

Several cross-sectional studies have found activity participation and differences in cognitive ability to correlate predictably among older adults. "Higher levels of participation in activities such as reading the newspaper predicted stronger memory, fluid, and crystallized cognitive abilities" (Bielak, 2009; Christensen et al., 1996). Moreover, having recurrent contact with individuals in a large social network, whom also provide greater emotional support, appeared to induce better performance on a test of general cognitive functioning (Holtzman et al., 2004). Although many other studies support these results (Bugg et al., 2006; Karp et al., 2006; Newson and Kemps, 2006; Parslow et al., 2006; Roth et al., 2003), some did not confirm a positive concurrent relationship (Hambrick et al., 1999; Salthouse et al., 2002; Sturman et al., 2005). One of these studies, conducted by Hambrick and colleagues (1999), found that individuals who spent more time working on crossword puzzles experienced less age-related decline in reasoning ability.

Compared to cross-sectional research, longitudinal studies provide a more stringent assessment of the engagement hypothesis by determining whether "baseline activity participation" (Bielak, 2009) reliably precedes or predicts changes in cognitive abilities over time. In fact, there is ample longitudinal support for a relationship between an engaged lifestyle and cognitive function. For example, engaging in activities such as, reading, playing chess, and completing crossword puzzles more frequently, ensures a more gradual 5-year decline in perceptual speed (Ghisletta et al., 2006).

Not only that, each extra hour spent on television viewing – "a poor provider of cognitive stimulation" (Bielak et al., 2009) – during middle adulthood increases the chance of developing Alzheimer's disease later in life by 30 percent (Lindstrom et al., 2005). Similarly, "low baseline numbers of social relationships" (Bielak, 2009), lack of an emotional support system, and little social integration in the community was related to poorer cognitive performance and greater decrease in cognitive function over a period of 4 years (Zunzunegui et al., 2003).

However, there is longitudinal evidence that does not provide support for the engagement hypothesis (Aartsen et al., 2002). Nevertheless, these studies are significantly less in number than those in support of the hypothesis, and most of the findings in opposition of the hypothesis were based on a particular activity domain (Ghisletta et al., 2006) or a particular time frame (Bielak et al., 2007), thus did not completely contradict the relationship between engagement and cognition. Furthermore, the discrepancies between the cross-sectional and longitudinal evidence may have been due to the substantial differences in the methodologies utilized across the studies. To be specific, different studies may have used different modes of activity assessment, activity durations and intensity levels, activity categorizations, and different cognitive domains used as an outcome measure, just to name a few. These differences may, in turn, have masked or altered relationships between activity and cognition (Bielak, 2009). However, the current evidence still contains considerable gaps in the sense that there is still no definitive proof of a positive relationship between activity participation and cognitive change (Salthouse, 2006, 2007). Therefore, although the engagement studies generally support the 'use it or lose it' hypothesis, the evidence cannot yet be considered as conclusive.

One gap in activity research is the fact that demographic variables may impact the activity-cognition relationship. First of all, increasing life-span may affect the cognitive effort required to complete an activity due to age-related cognitive decline and mounting harmful effects from lifestyle choices and the environment (Bielak, 2009). In turn, the benefits obtained from one activity may vary at different points in life-span (Salthouse et al., 2002). Indeed, the impact of an engaging lifestyle appears to be enhanced in later adulthood compared to young or mid-old adulthood (Hultsch et al., 1993). However, a number of studies have shown that age differences in the activity-cognition relationship are equivocal. Salthouse and colleagues (2002), for one, demonstrated that cognitively engaging activities do not reconcile age-related differences in cognitive performance in individuals older and younger than 50 years of age. Further, Newson and Kemps (2006) failed to find any age-related differences in the benefits obtained from cognitive engagement across early and late adulthood (i.e. 18–27 years; 65–92 years), but did find age-increasing benefits when individuals participate in physically demanding activities. Clearly, the existing data on the relationship between activity participation and cognitive function across the life span is still inconclusive at this point. Thus, Salthouse (2008) recently made a request for an increase in research on several different points in life span (e.g. every ten years instead of only early adulthood and late adulthood), as "we may be missing key information by overlooking the fact that a large amount of cognitive decline has already occurred by age 60". Furthermore, other demographic variables possibly influencing the relationship between activity participation and cognitive functioning are contextual factors, such as retirement or widowhood (Hertzog et al., 1999), which, in turn, tend to be influenced by gender or education.

Moreover, the benefits of an engaged lifestyle may also be limited to low-functioning individuals, as several studies have failed to find significant relationships between activity participation and cognition in highly educated and intelligent participants (Salthouse et al., 2002). To begin with, Scarmeas and colleagues (2001) demonstrated adults with both low levels of education (i.e. 8 years or less) and activity participation to have the greatest risk of developing Alzheimer's disease. Furthermore, Christensen et al. (1996) found that lower-educated elderly individuals, who often read the newspaper and actively pursued their interests and hobbies, had significantly higher memory scores than their more sedentary counterparts, whilst a similar activity level made little difference for highly-educated elderly individuals. In spite of this, other studies did find activity engagement level and cognition to be positively and significantly correlated in highly educated aging adults (Bielak et al., 2007; Wilson et al., 2005; Hultsch et al., 1999), suggesting that improvements in cognitive function are feasible regardless of educational level. However, as both activity participation and educational attainment are

thought to affect cognitive reserve (Kramer et al., 2004), the possibility exists that the cognitive benefits reaped from activity participation are less in highly educated adults because they already have accrued significant neural reserve from education.

Furthermore, cognitive gain from activity engagement has also been found to vary in relation to gender. Although the extent of participation in activities involving observation and investigation of "physical, biological, and cultural phenomena" (Bielak, 2009) was found to be predictive of superior cognitive speed scores in younger, middle-aged, and older men, in women this was only the case for middle-aged counterparts (Parslow et al., 2006). On the contrary, frequent participation in "intellectual-cultural activities, such as reading, listening to the radio, and having social visits" (Bielak, 2009) in early and middle adulthood protects women from developing dementia, but not men (Crowe et al., 2004). In line with this, active interaction with friends helped prevent cognitive decline in Spanish women, whilst men remained unaffected (Zunzunegui et al., 2003; Béland et al., 2005). As these were older women, the majority probably did not have jobs outside the house, whilst the husbands did. This, together with the fact that the brains of women are already instinctively wired to place more importance on social interactions than men, was most likely the reason why mental stimulation obtained from leisure activities may have had more profound effects on women than men, who are already satiated by work-related intellectual stimulation.

Even though the results generally prove that an engaging lifestyle, in spite of the demographic data variations, delays the onset of AD, the results must still be interpreted with caution. This is because, first of all, there is still no agreement on the best method of assessing activity participation. Although activity assessments usually "ask older adults to report their typical frequency of engagement in a specific list of activities" (Bielak, 2009), the activities specified and the number assessed tends to vary greatly. This makes sense, as no one, at this point, has investigated whether assessing more activities (e.g. fifty) rather than less (e.g. five) is more effective for obtaining clear-cut, reliable data (Bielak, 2009). Nevertheless, it can probably already be estimated that assessing a large number of activities will be more valuable than assessing a small number of activities, as brief activity questionnaires might potentially miss important activities that influence cognitive functioning.

Secondly, as the most common method used for assessing activity engagement, self-report, has a number of potential biases, the results must be interpreted with caution for these reasons as well. To begin with, one bias lies in the fact that participants "may answer activity questionnaires in a more (socially) desirable way" (Bielak, 2009), and therefore over-exaggerate how physically active they really are (Salthouse, 2006). Salthouse and colleagues (2002) found evidence of this bias when a number of participants had estimated their weekly activity (excluding sleeping) to equal an unrealistic 170 hours. Moreover, participants may also forget to report an activity, especially if they do not routinely partake in these activities (Dishman, 2006). Another bias is encountered when the influence of early life activity engagement on cognition is assessed using retrospective self-reports of leisure activity (Bielak, 2009). In other words, when subjects have to report activities dating many years back, the accuracy of the reports becomes dubious, especially as episodic memory has been found to regress with age (Craik, 2000). At the same time, however, it is possible that "today's older adult cohorts" (Bielak, 2009) had far fewer leisure activities at their disposal in earlier periods of their lives (e.g. television, computer-related activities), making them easier to remember. However, even research focused on the impact of more recent activity on cognitive function is subject to measurement error, as substantial changes in activity participation (due to e.g. illness, travelling, relocation) can occur over short and long periods of time, and are often not recorded by the questionnaires (Bielak, 2009). Thus, both distant and near retrospective studies can report inaccurate relationships with cognition.

Another reason why the data must be interpreted with caution, is because the cognitive domains used to assess potential associations with an engaged lifestyle tend to vary from one study to the next, making the identification of a consistently significant domain more difficult (Bielak, 2009). And as particular activities may enhance only specific cognitive abilities, studies neglecting to assess those cognitive domains may cause them to incorrectly conclude that the activity type had no effect on

cognition (Small et al., 2007). Therefore, the significant relationships identified, may, in fact, be side-effects of the cognitive domain selection used to evaluate the hypothesis.

Fourthly, failure to control confounding variables in lifestyle engagement studies may also lead to inaccurate conclusions. In research, education and socioeconomic status are generally statistically controlled for (Newson and Kemps, 2005), as these factors are known to have an effect on cognitive functioning. However, a key influencing factor that is often overlooked is sensory functioning (Newson and Kemps, 2005). Altered sensory functioning in elderly individuals is often based on age-related changes in the central nervous system, particularly in the biological indicators of aging, such as vision and hearing (Baltes & Lindenberger, 1997; Hofer, Berg & Era, 2003). These changes in sensory functioning will determine how optimally elderly individuals will participate in activities, as their functional capacity is restricted, and therefore their cognitive functioning as well (Anstey & Smith, 1999; Spirduso, 1995).

The last point addresses the final factor that may possibly confound the data demonstrating a positive relationship between an engaging lifestyle and cognition. Although the engagement hypothesis predicts that activity participation precedes and therefore causes subsequent changes in cognitive functioning in older adulthood, support for the opposite relationship, where changes in cognitive performance result in changes in activity participation, also exists. In the latter case, activity is said to be a marker of deterioration, rather than the underlying mechanism, and withdrawal from activities is thought to be the consequence, instead of the antecedent of cognitive regression (Mackinnon et al., 2003). According to this notion, individuals who have experienced cognitive decline may withdraw from activity participation because they find that the same activities they could partake in easily in the past, are now more difficult and a source of frustration. On the contrary, however, individuals with more superior cognitive functioning are able to engage in activities more effectively, and may therefore be more motivated to engage in future activities. Thus, although the 'use it or lose it' hypothesis is clearly in line with the notion of "differential preservation" (Bielak, 2009), used to describe the deviations in the levels of cognitive functioning with increasing age as a result of differences in activity level, at the same time, several studies have indicated that "preserved differentiation" (Bielak, 2009) is also key to the hypothesis, as active individuals likely always had superior cognitive abilities. This sustained, higher cognitive status enables them to engage in activities more frequently at older age (Salthouse et al., 1990; Salthouse, 2006), in turn, increasing the likelihood that these individuals will reap the benefits from 'using' their brain.

iv. The effects of environmental enrichment in mice

Since the physiological and cellular effects of an engaged lifestyle have not been widely investigated in humans, the next section of the chapter will focus on changes in mouse brains as a result of environmental enrichment (EE), solely because these studies most closely mimic the conditions of lifestyle engagement in humans. As was mentioned before, an 'enriched environment' for mice consists of several components, such as expanded learning opportunities, social interaction and physical activity (Fernandez et al., 2004), encouraged by placing toys and running wheels in their cages. Even though the previous section treated exercise separately from mentally stimulating activities, the next part will not dissociate between the two, as EE in mice tends to encompass both.

Although researchers predicted environmental enrichment to alter synaptic function, as connections between neurons have been shown to change in response to new environments, they did not expect AD pathology to be affected in APP transgenic mice. Lazarov and colleagues (2005) had placed nine of these mice in 'enriched' cages for five months and found that these mice had higher levels of the Aβ-destroying enzyme, neprilysin (NEP), compared to the mice in normal cages *(figure 4)*. DNA analyses also revealed that a number of genes were upregulated in the enriched group, including genes that encode proteins involved in learning and memory, vasculogenesis, neurogenesis, nerve cell protection, and molecular mechanisms to isolate and remove Aβ. Moreover, neurons in the hippocampi of these mice are more resistant to death and neurogenesis is increased as a result of an increase in nerve growth factor (NGF) (Mattson, 2004). However, as the most physically active mice

in the enriched cages had the most dramatic reductions in Aβ and deposits, it suggests that an active brain in combination with an active body, might be key in reaping the benefits of an enriched environment. However, since exercise and mental stimulation were investigated together, it is not clear which factor contributed to what extent to the changes in the brains of enriched mice. On the whole, it appears as though the enriched environment has a protective effect; preventing Aβ peptide from reaching detrimental levels, instead of dissolving the already existing amyloid plaques. However, more experiments with larger numbers of animals are needed to determine exactly how enriched environments benefit mice, whether through increased physical activity, a boost in visual, social, and spatial stimuli that awaken the brain, or some combination of all of these factors.

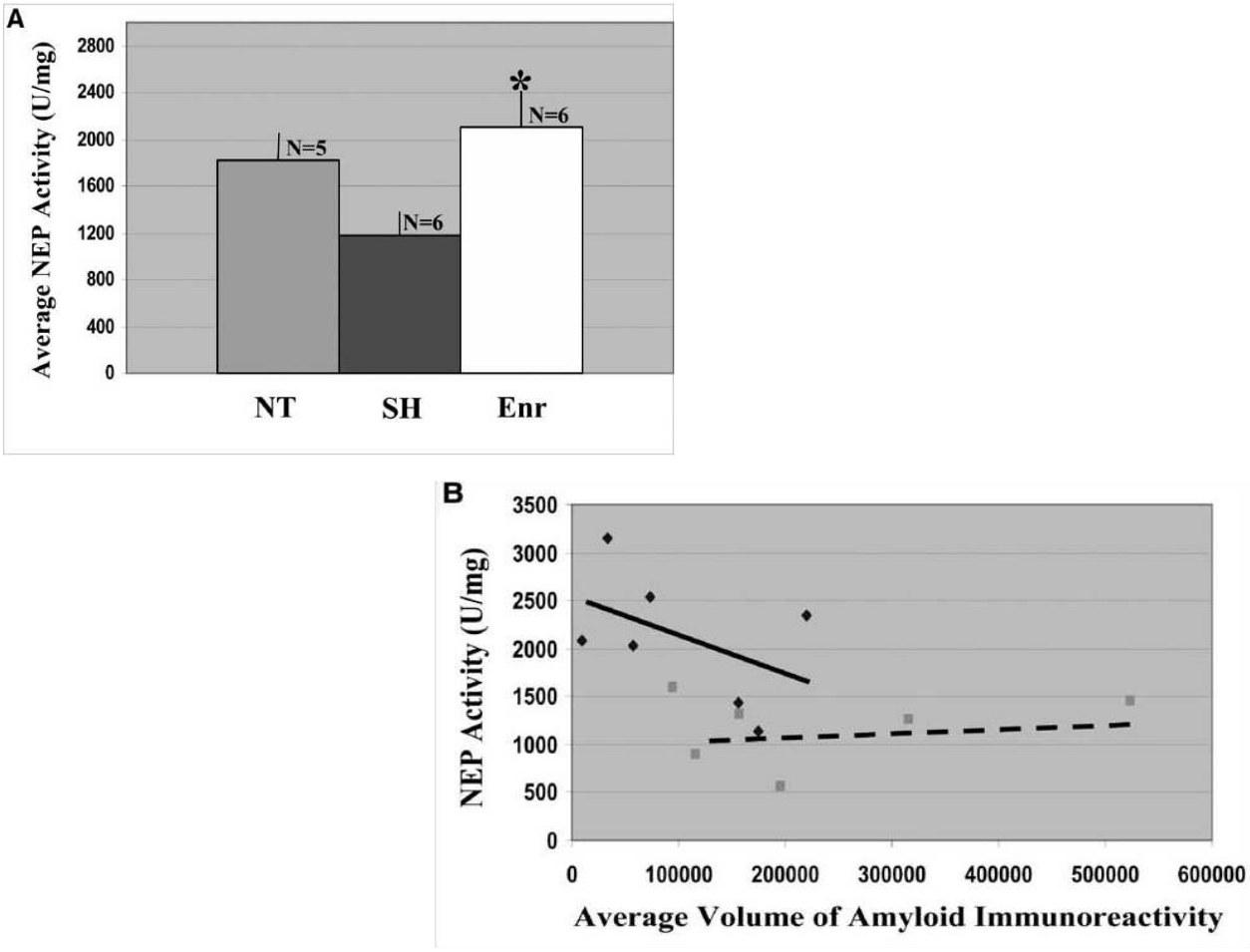

Figure 4. **Elevated neprilysin activity in brains of mice living in enriched housing conditions**.
A. Average NEP activity in the brain of nontransgenic (NT), enriched (Enr), and standard housing (SH) mice (mean ± SE; ANOVA, p % 0.0128). NEP activity is increased in the brains of mice that were kept in enriched housing conditions, compared to mice exposed to standard housing conditions.
B. NEP activity as a function of amyloid deposition. Amyloid burden and NEP activity form an inverse relationship in the brains of mice exposed to enriched housing conditions (standard housing = dashed line; enrichment = solid line).

v. The effects of environmental enrichment on oxidative stress

As was described before, ROS-induced oxidative stress appears to be a key feature of Alzheimer's disease. Studies have demonstrated that environmental enrichment can also protect against oxidative stress in Alzheimer-diseased brain. Female TgCRND8 mice kept under enriched housing conditions from day 30 until 5 months of age had "attenuated pro-oxidative processes and increased anti-oxidative defense mechanisms, as indicated by diminished biomarkers for reactive oxygen and nitrogen species, down-regulation of pro-inflammatory and pro-oxidative mediators, decreased expression of pro-apoptotic caspases, and upregulation of antioxidants SOD1 and SOD2" (Herring et al., 2010). Another study found environmental enrichment to specifically increase the production of glutathione in mice (Mattson et al., 2001). Together with the data from solely physically active mice, these studies therefore identify an antagonizing effect of environmental stimulation on Alzheimer's disease-related oxidative damage.

In sum, although an engaging lifestyle has not yet been shown to decrease Aβ deposition in humans, the fact that environmental enrichment decreases this peptide and upregulates antioxidant systems in the brains of mice is an exciting finding, as it is still completely relevant to the AD and aging process described in this review. Also, as most findings of mouse studies can be, to some degree, extrapolated to humans, it would not be completely illogical to expect that the improvements in cognitive function in humans as a result of an engaged lifestyle are due to similar changes.

Conclusion

Although research parties are still divided on some findings related to the etiology of Alzheimer's disease, it would be safe to say that soluble Aβ, oxidative stress and mitochondrial dysfunction as a result of aging, are enhanced in Alzheimer's disease. Moreover, for years now, it has been claimed that 'using' your brain will delay and attenuate the pathological and cognitive defects associated with AD. Although this review by far does not cover all the ways in which the 'use it or lose it' theory might be applicable, it did investigate the brain defects which studies found to be most prominently associated with Alzheimer's disease. Not only that, the last chapter was able to tie some of these defects to the 'use it or lose it' theory, as physical exercise and an engaging lifestyle result in changes in both the direct pathology of AD and the mechanisms that could potentially offset the defects, resulting in improved cognition and thus, most likely a delayed onset of Alzheimer's disease.

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