Introduction

‘Wear and tear’ versus ‘use it or lose it’

Several theories on the mechanism of aging and Alzheimer’s disease are based upon the assumption that during life, a continuous ‘wear and tear’ of the organism takes place. In that concept, increased metabolic activity would result in accelerated cellular aging. Analogies have been drawn in this connection between biological aging and the wearing out of shoes, clocks, piston rings and rubber bands due to sustained friction and oxidation (Swaab, 1991). The free radical theory of aging (Harman, 1981; Sohal, 1993) has provided a plausible mechanism to explain ‘wear and tear’ and there is indeed evidence for such a principle causing insults in cellular components such as proteins or the genome that are often irreversible (Gensler and Bernstein, 1981; Benzi and Moretti, 1995; Smith et al., 1995). According to the ‘wear and tear’ hypothesis, this damage would accumulate with age and cause progressive mal-functioning and, ultimately, the death of the cell. Well known examples that support this concept are e.g. studies on flies that revealed an inverse relationship between life-span and metabolic rate. Life-span could be prolonged considerably by reducing the level of physical activity and thus the metabolic rate of these flies (Sohal and Donato, 1979; Orr and Sohal, 1994; Fig. 1). This effect of physical activity was indeed related to enhanced oxidative DNA damage levels as measured in homogenates of these insects (Agarwal and Sohal, 1994; Orr and Sohal, 1994), suggesting that oxidative damage as a consequence of physical activity is a major causal factor in physical aging. In addition, courtship, which also increases physical activity, reduces the longevity of male Drosophila melanogaster (Cordts and Partridge, 1996). Oxidative damage is thought to be an early event in Alzheimer’s disease (AD) (Nunomura et al., 2001), and APOE isoforms have different anti-oxidant activities with APOE-ε4, a major risk factor for AD, showing the lowest protective activity (Tamaoka et al., 2000). In nuclear DNA of peripheral lymphocytes from AD patients, elevations of oxidized purines were observed, both at the basal level and following oxidative stress induced by H2O2. AD patients also showed a diminished repair of H2O2-induced oxidized purines (Mórocz et al., 2002).

On the other hand, the ‘wear and tear’ concept does not tally with the therapeutical advice that is generally given to old people or early Alzheimer patients, viz. to stay active and stimulate the brain. Lorand (1913) already stated in 1913: “work of any kind, even mental work alone, is a means of preventing precocious senility”. Millard (1984) worded
the same concept: “until better evidence is available I think I shall tell my mother to go on doing the crossword: like other organs may not brains deteriorate with disuse?”. Comparative studies have made it clear that the maximal life span and hence the rate of aging is not only inversely related to the basal metabolic rate, as predicted by the free radical theory, but is also directly related to the evolutionary increase of the brain relative to body size (Sacher, 1976, 1978; Hofman, 1993). A number of neuropathological observations support the idea that smaller brains age more rapidly. Down’s syndrome is accompanied by a smaller brain size (Wisniewski and Damoska, 1992; Tol et al., 1999; Pinter et al., 2001) and a shorter life expectancy (Thase, 1982; Dupont et al., 1986; Baird and Sadovnick, 1987). AD, for which aging is the major risk factor (Tol et al., 1999) occurs in all Down’s syndrome patients already before the age of 50 years (Ropper and Williams, 1980; Raghavan et al., 1994) and menopause occurs 4 years earlier (Seltzer et al., 2001), indicating premature aging of the brain in this disorder. Neuropathological lesions typical for AD are also prevalent among non-Down’s syndrome mentally retarded adults (Popovitch et al., 1990). Alzheimer’s neuropathology was also observed in a case of a 35-year-old mentally retarded patient with Williams’ syndrome (Golden et al., 1995). An extreme form of premature aging as judged from a decrease in brain weight and life span is found in micrencephalics. A decline in brain weight occurs after 3–5 years of age, while head circumference remains unaltered (Fig. 2). More than 85% of the males and 78% of the females die before they reach the age of 30 years (Hofman, 1984). No information on Alzheimer’s neuropathology is, however, available in micrencephalics.

There is also an increasing amount of literature indicating that activation of neurons may have a beneficial effect on neuronal function and survival during aging and AD and as a mechanism for this phenomenon the ‘use it or lose it’ principle has been proposed (Swaab, 1991; Swaab et al., 1998).

Having a high IQ also helps to survive longer (Whalley and Deary, 2001). The positive correlation found between the age of onset of AD and premorbid brain size suggests that brain size may be an important determinant for the occurrence of AD symptoms (Schrofield et al., 1995, 1997). Furthermore, the intelligence of AD patients is positively correlated with premorbid brain volume and negatively with the magnitude of brain atrophy (Mori et al., 1996). A smaller head circumference, an indication for smaller brain size, hastens the age of onset of AD and goes together with a longer disease or more rapid progression of AD (Graves et al., 1996; Borenstein-Graves et al., 2001; Gatz et al., 2001), suggesting that a larger brain may provide protection against AD or is a determinant of reserves. Additional relevant observations are the associations reported between low education level and poor performance on mental status examinations that were found in AD (Mortimer and Graves, 1993). Several
studies have indicated that education may protect against dementia (Fratiglioni et al., 1991; Kondo et al., 1994; Stern et al., 1994; Bonaiuto et al., 1995; Mortel et al., 1995).

The ‘use it or lose it’ principle might also apply to other neurodegenerative diseases, as indicated by a study by Kish et al. (1992b) who found a negative correlation between dopamine loss during aging and neuronal activity. A significant decline was present in striatal dopamine levels with increasing age. The level of dopamine metabolism in different parts of the striatum, however, as measured by the homovanillic acid/dopamine ratio, was found to be inversely related to the degree of dopamine loss. This suggests that striatal subdivisions with a physiologically higher dopamine metabolism run less
risk of suffering from dopamine loss with advancing age. Similar relationships have been reported for nonhuman primates treated with the neurotoxin MPTP, in which the most severe dopamine loss was found in subdivisions of the striatum with the lowest dopamine turnover rates (Elsworth et al., 1989). These data do not tally with the oxidative damage hypothesis of aging (Ames, 1989; Harman, 1994) and support the ‘use it or lose it’ concept. In conclusion, at present there are data indicating that stimulation of the brain may slow down brain aging and diminish the risk for neurodegenerative diseases, such as AD and Parkinson’s disease. This idea will be worked out in the present review.

Alzheimer’s disease

Neuropathology

Alzheimer’s disease (AD) is a multifactorial disease in which age and APOE-ε4 are important risk factors. AD occurs in 94% in patients over the age of 65 years and its prevalence is exponentially increasing with age. The presence of an APOE-ε4 genotype is associated with memory decline in cognitively impaired elderly (Dik et al., 2000) prior to the clinically symptomatic phase of dementia (Jonker et al., 1998). APOE-ε4 is responsible for about 17% of all AD patients (Tol et al., 1999), has a strong effect on the prevalence of AD neuropathology (Polvikoski et al., 2001) and is accompanied by a significantly greater rate of volumetric loss of the hippocampus of non-demented elderly than found in ε4 negative individuals (Moffat et al., 2000). AD is characterized histopathologically by the presence of large numbers of neuritic plaques (NPs) and cytoskeletal changes that are present as silver staining neurofibrillary tangles (NFT). NFT are present in the cell bodies of affected neurons, while the same cytoskeletal changes are called neuritil threads when present outside neuritic plaques (Braak et al., 1986) or dystrophic neurites when they are the neuritic components of neuritic plaques (Kowall and Kosik, 1987). Dystrophic neurites or neuropil threads are short, thickened, curly, coiled or sometimes hooked fibers. To a lesser degree, neuritic plaques and cytoskeletal changes can also be observed in aged, non-demented control subjects. The APOE-ε4 allele is associated both with plaques and tangles in a sex- and age-related way (Ghebremedhin et al., 2001).

It is a well-accepted fact now that the neuropathological AD changes are not restricted to the cholinergic system, but also involve all other types of transmitter systems, i.e. also amines, amino acids and peptides. It is also well-known that the AD pathology is not restricted to the cortex and hippocampus, but also affects subcortical regions (Braak and Braak, 1995). In addition, the neuropathological changes cannot be distinguished qualitatively from those appearing in elderly non-demented subjects. Finally, AD is not a monocausal disease, except for some relatively rare familial cases, but a multicausal disorder with age and APOE-ε4 as the most important risk factors (Tol et al., 1999).

Not simply amyloid accumulation as the cause of AD neuropathology

Although AD is neuropathologically characterized by plaques and tangles, it is still a controversial issue whether these hallmarks are responsible for the clinical symptoms of dementia or a consequence of the disease. We have provided evidence (for review see Swaab et al., 1998) for a number of arguments against the amyloid cascade as the pathogenetic mechanism in AD, i.e., that amyloid accumulation would be toxic, lead to tangles and subsequently to cell death that would be the cause of dementia.

(1) It is very improbable that the pathogenetic process can be explained by a cascade starting with amyloid (β/A4) deposits as suggested e.g. by Selkoe (1994). The first signs of AD are NFT occurring in the entorhinal cortex, quite often preceding the presence of amyloid plaques (Braak and Braak, 1991, 1995). In the nucleus basalis of Meynert, amyloid deposition rarely occurs in the early stages of AD, whereas neurofibrillary changes are consistently present (Sassin et al., 2000). The existence of amyloid deposits can, thus, not be considered as a prerequisite for the development of NFT.

(2) Moreover, the neuropathological hallmarks of AD do not follow each other up in the course of time as one would expect on the basis of the amyloid cascade hypothesis but are basically independent phenomena. There are brain regions with almost only neurofibrillary changes and no amyloid and vice
versa (Van de Nes et al., 1993; Sassin et al., 2000). Studies in transgenic mice did not reveal evidence for the idea that the amyloid precursor protein (APP) or Aβ would induce neurofibrillary tangles. However, recent studies in double mutants and injection of Aβ showed that the neurofibrillary pathology can be significantly enhanced by overexpression of APP or Aβ (Götz et al., 2001; Lewis et al., 2001). Yet, also in these models, tangle development did not occur at the same site where Aβ was injected and the classic AD plaques with a corona of tau-positive dystrophic neurites was not found in these mice (Lee, 2001).

(3) Cell death in AD is not a major generally occurring phenomenon. Although the neocortex in AD is atrophic (Regeur, 2000), the total number of neurons is not diminished (Regeur et al., 1994; Fig. 3). We have shown that cortical neurons containing hyperphosphorylated tau deposits can still be viable (Verwer et al., 2001). Cell death is restricted to a few brain areas, i.e. the CA1 area in the hippocampus (West et al., 1994), the entorhinal cortex and the superior temporal gyrus (Gómez-Isla et al., 1996, 1997) and the locus coeruleus (Bondareff et al., 1982; Chan-Palay and Asan, 1989; German et al., 1992; Hoogendijk et al., 1995).

(4) A long struggle has been going on about the question what is more important for the development of the clinical signs and symptoms of dementia and cell death: cytoskeletal changes or amyloid deposits. The answer is: probably neither. Since the CA1 area of the hippocampus shows a massive number of NFTs and NPs in AD and is one of the few areas that show a very clear neuronal loss in AD as shown by West et al. (1994), we chose this brain area to study the question whether or not NPs may induce local cell death as presumed by many authors (Emre et al., 1992; Kowall et al., 1992). Our study (Salehi et al., 1996) showed that there is indeed a slightly lower neuronal density around NPs. In addition, we found a negative relationship between the size of the neuritic plaques and neuronal density around them, indicating that the neurotoxic effect is dose-dependent. However, the contribution of this neurotoxic effect on the total cell death in the CA1 area was much lower than generally presumed and, in fact, extremely limited, i.e. 2.6% out of the reported 70% cell death. This study therefore supports the notion that the occurrence of NPs and cell death are largely two independent phenomena.

(5) A greater loss of synapses than neurons was found in the cortex of AD patients (Davies et al., 1987). As shown by Terry et al. (1991) and Terry (2000) loss of synapses is proposed to lead to cognitive decline. But here, too, a loss of markers for active synapses such as synaptophysin or microvesicles may have been determined instead of a loss of synaptic contacts. In addition, synaptic pathology in prefrontal cortex is only present in severely demented AD patients and not in the mild/moderate cases (Minger et al., 2001). As will be argued in this review, reduced neuronal activity is most probably one of the major characteristics of AD that occurs early in the disease process and may underlie the clinical symptoms of dementia (see below). The consequence of this is that it is attractive to direct therapeutic strategies towards restimulation of neuronal metabolism in order to improve cognitive and behavioral symptoms of AD.

Neuronal atrophy rather than cell loss in many brain areas in aging and AD

During normal aging, cell loss is not a prominent phenomenon. In fact, unaltered neuronal numbers have been reported in many brain areas (Wickelgren, 1996) and the loss of neocortical neurons during lifespan was estimated to be only 10% (Pakkenberg and Gundersen, 1997). Regeur et al. (1994), using unbiased sampling and counting methods, showed that in spite of the generally observed cortical atrophy in AD, global neocortical neuronal loss does not take place in this brain area in AD patients (Fig. 3), providing strong evidence that neuronal shrinkage rather than cell death is a major phenomenon in this neurodegenerative disorder (Fig. 3).

Age-related memory disturbances and the loss of memory in AD have been related, at least partly, to cholinergic dysfunctions and degenerative changes in the nucleus basalis of Meynert (NBM). Neurotoxic lesions of the cholinergic system in experimental animals induce performance deficits. The selective destruction of NBM cholinergic cells impairs the ability of the neocortex to attend to and process short, highly salient sensory stimuli (Wenk, 1997b). Large reductions in cholinergic markers have been found
Fig. 3. Total number of neocortical neuron number in 11 AD patients and 10 matched controls. (From Regeur et al., 1994, with permission.) Note that there is no significant decrease in neuron number in the AD patients, excluding massive neuronal death as a major phenomenon in the cortex of AD patients.

in the cerebral cortex to which the NBM projects, even at an early stage of AD in biopsies (Bowen et al., 1982). Moreover, the number of choline acetyltransferase and vesicular acetylcholine transporter neurons correlates significantly with the severity of dementia, as determined by the mini-mental state examination test (Gilmor et al., 1999). Choline acetyltransferase activity in the medial, frontal and inferior parietal cortex of AD patients correlates with praxis scores, and medial frontal acetylcholinesterase activity correlates significantly with attention/registration scores. Cholinergic deficits may also contribute to behavioral disorders in AD patients (Minger et al., 2000). In spite of all those observations, neuronal loss in the NBM is not as extensive as was presumed earlier (see below).

**Neuronal loss versus atrophy in the NBM**

The evolution of AD-related cytoskeletal changes has been described by Sassin et al. (2000). The initial cytoskeletal abnormalities are already seen in Braak stage I, while amyloid deposits rarely occur. Subsequently a neurofibrillary tangle is formed as a spherical somatic inclusion in this brain structure. Finally, the cell may die, leaving behind an extraneuronal ‘ghost tangle’. Estimations of the neuronal numbers of the NBM during normal aging vary
greatly, i.e., from losses ranging from 23 to 90% (Mann et al., 1984; McGeer et al., 1984; Etienne et al., 1986; Lowes-Hummel et al., 1989; Cullen et al., 1997) to no significant neuronal loss at all (Whitehouse et al., 1983; Chui et al., 1984; Bigl et al., 1987).

Massive cell death in the NBM was originally presumed to be one of the major hallmarks of AD (Whitehouse et al., 1981, 1982; Arendt et al., 1983; Mann et al., 1984; Etienne et al., 1986) and a clear loss of the markers of NBM neurons, choline acetyltransferase, was reported (Pearson et al., 1983). However, it is of crucial importance to distinguish a loss of cholinergic markers from a loss of neurons, at least for the late phases of AD, since in patients with early signs of AD no change in choline acetyltransferase or acetylcholinesterase were observed in neocortical areas (Davis et al., 1999). It has been presumed that the large differences in cell loss that were reported, may, at least partly, be due to the heterogeneity of the different subdivisions of the NBM (Iraizoz et al., 1991). Indeed, Vogels et al. (1990) found an overall neuron loss in the NBM of only 10%, while neuron loss varied from 0% in the rostral to 36% in the caudal part of the NBM. However, regional heterogeneity cannot be the only explanation for the variable data reported. Even studies performed on one particular NBM subdivision showed a considerable variation. For instance, measurements performed in the NBM subarea Ch4a showed differences varying from a cell loss of between 42 and 89% (Mann et al., 1984; Cullen et al., 1997) to no significant cell loss at all (Pearson et al., 1983). Gilmor et al. (1999) studied the NBM in patients without cognitive impairment, mild cognitive impairment and early stage AD, using choline acetyltransferase and the vesicular acetylcholine transporter as markers for the NBM neurons. No significant difference was found between the three groups and only a 15% non-significant reduction in the number of NBM neurons was found in the early AD cases, showing that certainly in the early stage of the disease, these neurons are relatively preserved.

The most likely explanation for the equivocal results concerning neuronal loss in the NBM in AD is the use of different criteria for the size of counted cells, which is crucial, considering the atrophy NBM neurons appear to undergo in AD. Mann et al. (1984), for instance, only counted cells with a diameter larger than 30 µ.m and reported a 54% cell loss in the NBM, whereas Pearson et al. (1983) counted all NBM neurons regardless of their size and did not find any significant cell loss in the NBM. Indeed, while the number of large neurons decreases, the number of small neurons increases in the NBM in AD (Whitehouse et al., 1983; Rinne et al., 1987; Allen et al., 1988; Vogels et al., 1990; Fig. 4). The combined data indicate that the majority of the large neurons atrophy and lose their cholinergic markers, and that only a small subset dies.

Metabolic activity in the NBM in relation to APOE genotype

For the reasons mentioned above, the general concept of major cell loss in the NBM of AD patients had to be abandoned and was replaced by the opinion that neuronal atrophy rather than cell death is the major hallmark of AD in the NBM (Pearson et al., 1983; Rinne et al., 1987; Salehi et al., 1994; Swaab et al., 1994, 1998). The size of the Golgi apparatus (GA) is a sensitive parameter for changes in neuronal metabolic activity. Both in animal experiments (Jongkind and Swaab, 1967; Swaab and Jongkind, 1971; Swaab et al., 1971) and in the human postmortem hypothalamus (Lucassen et al., 1993, 1994; Ishunina et al., 1999) it has shown to be a valuable measure for changes in neuronal activity, independent of neurotransmitter or neurohormonal content, and independent of species or type of pathology (Mourelatos et al., 1993; Dal Canto, 1996; Stieber et al., 1996, 1998). In addition, the GA marker thiamine pyrophosphatase was significantly reduced in the cortex of AD patients (Raghavendra Rao et al., 1993), again illustrating the usefulness of the GA as a marker for neuronal activity. GA size was, therefore, used in our studies to monitor activity changes in the NBM in aging and AD.

A strong decrease in GA size was observed in AD (49%) (Fig. 5), suggesting that the capacity of NBM neurons to process and target proteins decreases dramatically in AD (Salehi et al., 1994, 1998). This conclusion is consistent with studies showing a decreased volume of the nucleolus as an index for the protein synthetic capacity of NBM neurons in AD (Tagliavini and Pilleri, 1983; Mann et al., 1984) and
Fig. 4. Size-specific numerical densities \( N_v \) of neuronal nuclei and perikarya in non-demented controls and AD patients in the nucleus basalis of Meynert (NBM). (From Rinne et al., 1987, with permission.) Note that the number of large neurons decreases, while the number of small neurons increases, which illustrates neuronal shrinkage in the NBM.

Fig. 5. Graph showing the size of the mean Golgi apparatus in controls and AD patients with ApoE genotype ε3/3 compared with AD patients with ApoE-ε3/4 and -ε4/4. The size of the Golgi apparatus is a measure of neuronal metabolism. Note the clear reduction in the size of Golgi apparatus in AD patients with one or two ApoE-ε4 alleles, compared with AD patients without ApoE-ε4 alleles. There is no significant difference \( (P = 0.760) \) in Golgi apparatus size between AD patients with one ApoE-ε4 allele and two ε4 alleles (From Salehi et al., 1998, with permission.)
agrees with earlier studies providing evidence for a decrease in the activity of the enzymes choline acetyltransferase and cholinesterase in the NBM in AD (Perry et al., 1982; McGeer et al., 1984; Etienne et al., 1986; Perry, 1986; Araujo et al., 1988). There is not only a strong reduction in neuronal metabolic rate in the NBM of AD patients, but also an extra reduction in AD patients with either one or two APOE-ε4 alleles (Fig. 5; Salehi et al., 1998). This finding is in full agreement with the more severe cholinergic deficit in the temporal cortex observed in AD patients with one or two APOE-ε4 alleles (Poirier et al., 1995). There are indications that APOE genotype in the long term may affect the response to anticholinesterase therapy in AD patients. APOE-ε4-positive women are the most likely patients to benefit (MacGowan et al., 1998), supporting the importance of this genotype for cholinergic functioning.

Postmortem temporal cortex tissue obtained from cognitively normal APOE-ε4 subjects had already lower cholinergic activity than tissue from subjects without this allele (Allen et al., 1997). Moreover, not only memory complaints but also APOE-ε4 allele carriage predict cognitive decline at an early stage (Dik et al., 2001). Therefore we are currently investigating the question whether the NBM of APOE-ε4-positive cognitively normal controls without AD pathology (i.e., Braak stage 0–II), neuronal metabolism is already lower than observed in APOE-ε4-negative subjects. Indeed, using the size of the GA as measure for neuronal metabolism in the NBM seems to be the case (E.J.G. Dubelaar et al., unpublished observations).

Neurotrophin receptors in the NBM

In the basal forebrain complex, both low affinity nerve growth factor (NGF) receptors (p75, Hefti et al., 1986; Allen et al., 1989) and high affinity NGF receptors (Kordower et al., 1989) are present. All three family members of the high affinity NGF receptors, the tyrosine receptor kinases (trks) A, B and C are found in NBM neurons (Muragaki et al., 1995; Shelton et al., 1995; Salehi et al., 1996). Both trk and p75-immunopositive neurons are already found in the NBM as early as embryonic week 14 (Chen et al., 1996). NGF in the NBM decreases during aging and even more so in AD (Hefti and Mash, 1989; Mufson et al., 1995). Studies from our group show that all three types of trks colocalize in the NBM neurons and decrease in AD, although trk-A decreases more than trkB and trkB decreases more than trkC (Salehi et al., 1996; Fig. 6). TrkA mRNA

![Graph depicting the proportion of neurons stained by trk antibodies in controls and AD patients in the nucleus basalis of Meynert. Note the strong reduction in the proportion of trkA-expressing neurons in AD, which is followed by trkB and trkC. * P = 0.00001, ** P = 0.08, *** P = 0.04. (From Salehi et al., 1996.)](image)
levels decrease markedly in AD (Mufson et al., 1996). The reduction in the expression of trkA has subsequently been confirmed (Boissiere et al., 1997; Mufson et al., 1997). Moreover, it was shown that a loss of immunoreactive trkA neurons already occurs in individuals with mild cognitive impairment without dementia, to the same degree as in early AD (Mufson et al., 2000). The reduction in trk receptors may underlie the diminished retrogradely transported NGF levels in the NBM, leading to their decreased metabolism and function.

In contrast, expression of the gene encoding for the low affinity p75 receptor was reported not to be significantly altered (Mufson et al., 1996). Also, based on Northern blot (Goedert et al., 1989) and receptor binding studies (Treonor et al., 1991) the expression of p75 in NBM neurons was reported to be unaltered in AD. These findings are not without controversy, since Arendt et al. (1997) found an APOE-ε4 related decrease in the number of p75 immunoreactive NBM neurons. We have quantified p75 immunoreactivity in the NBM of 31 controls and 30 AD patients and their matched controls and observed a significant decrease in p75 staining, both in cell bodies and in fibers. The fibers in the NBM contained even less p75 in younger AD patients (Salehi et al., 2000).

It thus seems that both high and low affinity neurotrophin receptors are decreased in the NBM of AD patients. In addition, a defect in retrograde transport of NGF to the NBM of AD patients has been observed (Mufson et al., 1995; Scott et al., 1995). This defect may be related either to the decreased amounts of trk receptors in AD (Salehi et al., 1996), to the decreased amount of p75 (Salehi et al., 2000) or to the cytoskeletal changes in the NBM (Swaab et al., 1992) that are presumed to hamper axonal transport (Swaab et al., 1992). It has been postulated that both types of neurotrophin receptors, the trks and p75, are transported anterogradely to the cortex and hippocampus. Upon binding to these receptors, neurotrophins are retrogradely transported back to the basal complex neurons where they provide trophic support. Exactly how decreased neuronal metabolic activity (Salehi et al., 1994), cytoskeletal changes (Swaab et al., 1992), the loss of trk and p75 receptors (Salehi et al., 1996, 2000) and the failed retrograde transport of NGF are related to the diminished function of the NBM neurons (Cooper et al., 2001) should be studied further.

In a pilot study using a radio-controlled fully implantable pumping device delivering NGF to the lateral ventricle of a 69-year-old female AD patient with symptoms of dementia for 8 years, increases in blood flow and nicotine binding in frontal and temporal cortex were noted, as well as a persistent increase in cortical blood flow as evaluated by positron emission tomography and improvement of the EEG and psychological tests (Olson, 1993; Seiger et al., 1993). However, these effects were only limited and short-lasting, as may perhaps be expected from the loss of neurotrophin receptors in the cholinergic system of AD patients (see above). Moreover, a few additional AD cases treated with low doses of nerve growth factor experienced several serious side effects, including pain and weight loss. The pain disappeared within a couple of days after stopping the NGF infusion, and was followed by weight gain (Nordberg, 1996; Eriksdotter Jönhagen et al., 1998). Clearly, more knowledge on the regulation of neurotrophin receptor production in the various brain areas and the changes in AD patients is needed in order to be able to perform rational therapeutic trials in this field.

A significant and extensive decline in the number and size of cholinergic NBM neurons was found in aged rhesus monkeys. The loss of staining NBM neurons was nearly completely reversed by human nerve growth factor gene delivery (Smith et al., 1999). We have to wait and see whether a similar gene therapy in AD patients as currently performed by Tuszynski et al. (2002, this volume), will not lead to the same side effects as were reported earlier, for NGF infusion, and to better results.

**Decreased neuronal activity is a major and early hallmark of AD**

Various observations indicate that decreased neuronal activity is an essential characteristic of AD, either as a risk factor or as a direct pathogenetic factor (Beal, 1994), while a high or enhanced neuronal activity would protect against the degenerative changes of aging of AD, an hypothesis we paraphrased as 'use it or lose it’ (Swaab, 1991). The report that the postmortem AD brain shows a lower
total amount of protein (Suzuki et al., 1965), a clear reduction in total cytoplasmic RNA (Bowen et al., 1977; Mann et al., 1981; Doebler et al., 1988), messenger RNA (Sajdel-Sulkowska and Marotta, 1984; Guillemette et al., 1986; Taylor et al., 1986), a smaller cell size, such as the somatostatin neurons in the cortex (Joynt and McNeill, 1984) and a small size of the neuronal Golgi apparatus (Salehi et al., 1994, 1995a,b) are all indications of decreased metabolic activity in AD. In the frontal cortex a decrease of 28% in the amount of mitochondrial DNA was found (Rodríguez-Santiago et al., 2001). The activity of cytochrome oxidase (CO), which constitutes the rate-limiting enzyme of the mitochondrial electron transport chain, was reduced in the frontal, temporal and parietal cortex and hippocampus of AD patients (Kish et al., 1992a; Simonian and Hyman, 1993, 1994; Chandrasekaran et al., 1994; Chagnon et al., 1995; Verwer et al., 2000). In addition, mRNA coding for subunit II was severely decreased in the hippocampus of AD patients (Simonian and Hyman, 1994). Since CO activity is tightly coupled with neuronal activity (Wong-Riley, 1989), the reduction in its activity in AD may possibly be explained by neuronal hypofunction or mitochondrial loss. The deficiency of this key enzyme also points to the occurrence of an hypometabolic process in aging and AD (Kish et al., 1992a; Chandrasekaran et al., 1994).

In AD, especially in temporal and parietal lobes, as shown by positron emission tomography (PET) (Hoyer et al., 1988; Kumar et al., 1993; Meneilly and Hill, 1993; Meier-Ruge et al., 1994; Swerdlow et al., 1994). In both mild cognitive impairment and in AD, metabolism reductions exceed the loss of volume of brain structures (De Santi et al., 2001) and appropriate corrections for atrophy showed that reduced glucose metabolism in PET reflects a true metabolic reduction per gram of tissue (Ibáñez et al., 1998). In AD a marked reduction of regional cerebral blood flow and cerebral hemoglobin oxygenation may occur during activation of brain function (Hock et al., 1997). In carriers of the Swedish Alzheimer amyloid protein (APP 670/671) mutation, the clearest change related to the development of clinical AD was a reduction of cerebral blood flow in the basal and temporal lobes (Julin et al., 1998). The changes in regional cerebral glucose metabolism as measured by PET in the temporoparietal, prefrontal and occipital cortex, were correlated with a change of the Mini Mental State Examination score in probable AD patients, suggesting that clinical deterioration and metabolic impairment are closely related (Mielke et al., 1994). In addition, a significant negative relationship between metabolism and the density of plaques was found in AD (Mielke et al., 1996). A 19–45% reduction in the cerebral metabolic rate (CMR) of glucose, but not of oxygen, was found in mild to severe AD patients (Hoyer, 1992, 1995a,b; Salmon et al., 1996). This phenomenon was suggested to relate to brain insulin action, to brain insulin receptor function (Hoyer et al., 1991; Hoyer, 1995a,b; Craft et al., 1996) or reduced synaptic functioning (Salmon et al., 1996). Also, diminished activities of enzymes active in glucose metabolism and ATP formation from other sources than glucose have been demonstrated in AD (Hoyer, 1992, 1995b). In this respect, it is interesting to note that isolated microvessels from the temporal cortices of AD patients showed decreased glucose metabolism, suggesting a global defect in brain energy metabolism (Marcus et al., 1989). Indeed, a 50–70% decline of glucose metabolism is found in the brain of AD patients, causing the ATP synthesis to be critically lowered (Meier-Ruge et al., 1994). The reduced blood–brain barrier and neuronal glucose transporters GLUT-1 and GLUT-3 in AD patients may play a crucial role in these metabolic changes (Kalaria and Harik, 1989; Simpson et al., 1994; Mooradian et al., 1997).

As to the issue of hypometabolism being an early event, an important observation is that of Foster et al. (1984), who were the first to demonstrate that a substantial decrease in cerebral glucose metabolism may precede cognitive impairment. The observation that metabolic decline is a very early sign of AD was supported by Small et al. (1995) and Reiman et al. (1996), who found that in late middle-aged, cognitively normal subjects who were homzygous for the APOE-ε4 allele, and thus at risk for AD, have already reduced glucose metabolism in the same region of the brain that is later affected in patients with probable AD. The early metabolic and cognitive decline are also APOE gene-dependent. In middle-aged and older non-demented persons with normal memory performance, a single copy of APOE-ε4
was associated with lower inferior parietal, lateral temporal and posterior cingulate metabolism. Such a decreased neuronal metabolism as measured by PET was predictive for cognitive decline after 2 years of longitudinal follow-up (Small et al., 2000; Reiman et al., 2001). Although these authors speculate that it is possible this way to identify pathologically affected but not demented subjects at risk for AD, and that serial imaging will assist in response monitoring during experimental treatments, its potential for clinical success remains to be demonstrated (Rapoport, 2000).

The effect of APOE type is region-specific. APOE-ε4 genotype seems to go together with lower metabolic rate in the temporoparietal region, but with an increased metabolic rate in the frontal region (Higuchi et al., 1997). Perfusion MRI showed an impairment in temporoparietal blood flow with high sensitivity and specificity in early AD. The perfusion impairment was unrelated to atrophy and thus showing a true functional decline (Bozzao et al., 2001). Reduction of regional cerebral glucose metabolism in later stages of AD is related to particular neuropsychological impairments (Haxby et al., 1988; Mielke et al., 1994; Slansky et al., 1995). It is presumed that cortical glucose hypometabolism in AD may reflect reduced synaptic activity (Salmon et al., 1996). The observations in the temporoparietal area, where the AD process starts, support the notion that AD may primarily be a hypometabolic disorder (Swaab, 1991; Swaab et al., 1998). It is, moreover, interesting to note that Parkinson patients with dementia show a global decrease in glucose metabolism similar to that in AD, i.e. with more severe abnormalities in the temporoparietal region (Peppard et al., 1992).

**Tangles and NPs do not cause decreased metabolic rate**

Since the finding of a decreased metabolic rate in affected brain areas in AD, the two major questions concerning the pathogenesis of AD have been: (1) whether the presence of plaques or tangles in AD are indeed related to decreased neuronal activity in various brain areas and, if that is the case; (2) whether these neuropathological AD hallmarks induce decreased metabolic rate or vice versa. Alternatively, the neuropathological AD changes and decreased metabolic rate could occur independently. Our research supports the latter possibility.

In order to be able to study the causality of the relationship between the presence of NPs and NFT in a brain area with decreased metabolic activity we compared metabolic activity of CA1 neurons that did contain NFTs with those that did not. There appeared to be no difference in the size of the Golgi apparatus between these two groups of neurons. Consequently, the presence of NFT does not seem to cause an extra decrease the general metabolic rate of a neuron (Salehi et al., 1995a). So although NFT and decreased metabolic activity are present in the same brain area, i.e. CA1, they do not seem to be directly causally related. This observation is in agreement with the study of Gertz et al. (1989) who showed that the presence of intraneuronal NFT in the CA1 area of the hippocampus is not related to another parameter of general metabolic activity, i.e. nucleolar or cell size. This does certainly not exclude the possibility that tangles may decrease the production of certain specific compounds. Indeed, Häntämä et al. (1996) have shown that cytochrome oxidase subunit III mRNA is decreased in tangle-bearing neurons.

Neuritic plaques (NPs) are considered by some investigators as later stages of amorphous plaques (Rozemuller et al., 1989). Because of extensive damage to the neuropil in the vicinity of NPs, they are also called ‘malignant’ plaques (Wisniewski and Wegiel, 1995). Although it is still a matter of controversy, many investigators believe that the β-amyloid content of the core of the plaques is neurotoxic (see before) and induces neural degeneration. On the other hand, unlike in the case of NFTs, there is no clear relationship between the number of NPs and the severity of dementia (see before) which makes a neurotoxic effect of plaques as a major pathogenetic mechanism in AD symptomatology questionable. If a plaque were indeed to contain neurotoxic compounds one would expect that the closer a neuron is situated to the plaque, the lower its metabolic rate would be. Our measurements do not support the idea of such a mechanism. There appeared to be no relationship between either the density of NPs or the distance of each NP to the metabolic activity of neighboring neurons (Salehi et al., 1998). Conse-
sequently, this finding does not support the possibility that neurotoxicity of plaques causes decreased neuronal metabolism in vivo but rather that metabolism and NPs are two basically independent phenomena.

**Neuronal reactivation is still possible in elderly subjects**

Other data in favor of the 'use it or lose it' hypothesis are provided by studies on the infundibular nucleus of the hypothalamus of postmenopausal women. Strong activational changes were found in neurons expressing estrogen receptor and substance-P mRNA as judged from the pronounced neuronal hypertrophy and the occurrence of larger and double nucleoli. Also marked increases in tachykinin gene expression were found in this nucleus in postmenopausal women (Rance et al., 1990, 1993; Rance and Young, 1991; Rance, 1992). These changes are likely due to the loss of negative estrogen feedback as a result of ovarian failure in women. Activated neurons have been reported to be still present in this nucleus of women of over 100 years of age (Ule et al., 1983), suggesting that the activated neurons indeed remain intact in old age. In elderly men some activation may occur due to reduced circulating testosterone levels (Ule et al., 1983; Rance et al., 1993).

Interestingly, recent information suggests that the postmenopausal activation of the infundibular (= arcuate) nucleus in women prevents the formation of AD changes in this area. The sex-specific argyrophilic neurofibrillary changes in the median eminence and infundibular nucleus as observed by Gallyas silver stainings and antibodies to abnormally phosphorylated tau (Fig. 7), occur in most men over the age of 60 years but are seldom found in women of the same age (Fig. 8; Schultz et al., 1996, 1997, 1999). The activation of the infundibular nucleus in postmenopausal women is much more pronounced than in men of the same age (Rance, 1992). This observation may, therefore, serve as an extremely good and spontaneously occurring example of the 'use it or lose it' concept. In addition, these observations show that it is quite possible to stimulate successfully a neuronal population in the second half of life.

**Brain reserve by environmental stimulation**

Stimulation of rats in an enriched environment enhances (relative) brain size (Rozenzweig and Bennett, 1996; Mirmiran et al., 1986). Following enriched environment housing conditions, neural plasticity was induced even in old rats in which small increases in cortical thickness were measured (Van Gool et al., 1987).

AD patients were found to have reduced non-occupational activities already in mid life compared with healthy control group members. The increase in time spent on intellectual activities from early adulthood (20–39 years) to middle adulthood (40–60 years) was associated with a significant decrease in probability to belong to the Alzheimer’s group (Friedland et al., 2001). Although it has been suggested that education may increase brain reserve by increasing the density of cortical synapses and thus delaying AD symptoms (Katzman, 1993; Stern et al., 1995), the exact mechanism of the effects of education and occupation on the brain clearly requires further research. Occupation was shown to be a stronger indication of risk for dementia than education (Bonaiuto et al., 1995; Mortel et al., 1995).

**Reactivation as a means of restoring neuronal function in AD: clinical studies and therapeutical consequences**

The present review showed that there is a clear reduction in neuronal metabolic activity in various brain areas in AD patients. Consequently, one may assume that restoration of the activity of neurons, either by pharmacological or non-pharmacological stimuli, would lead to diminishment of cognitive impairment (Swaab, 1991; Swaab et al., 1998). Although it is not yet clear whether decreased metabolic activity is the primary process in the pathogenesis of AD, recent data show that reactivation of neurons is, in principle, beneficial to AD patients.

**Pharmacological stimuli**

One of the neurotransmitter systems clearly affected in AD is the cholinergic system (see before). Cholinesterase inhibitors enhance acetylcholine content in the synaptic cleft, which results in restora-
tion of cholinergic nicotinic receptor functioning and glucose metabolism. The application of moderately long-acting cholinesterase inhibitors such as tacrine (tetrahydroaminoacridine, THA), indeed has some positive effects on cognitive functioning in some AD patients and has even been claimed to slow down the course of the disease (Nordberg et al., 1992; Nordberg, 1995). The improvements in neuropsychological performance are paralleled by an increase in glucose metabolism and nicotinic receptors following tacrine treatment (Nordberg, 1995). Thus even transmitter replacement may fit in the
Fig. 8. The percentage of male individuals affected by the MBH pathology markedly increases from the age of 60 years to the age of 90 years. A marked or severe degree of the MBH pathology was identified in 30% of all males at this age (not shown). In contrast, only a small percentage of elderly females is affected. (From Schultz et al., 1997, Fig. 3a.)

The idea that enhanced functional brain activity can be obtained in AD after application of the proper stimulus. The positive effects of tacrine were mainly obtained in mildly to moderately demented patients, suggesting that a too strong hippocampal atrophy or neuronal loss prevents the beneficial effects of such a treatment (Riekkinen et al., 1995). A similar observation was made with transcutaneous electrical nerve stimulation (TENS) (see below). This illustrates that restimulating effects of a therapy may only hold if some ‘functional reserve’ in the form of critical amounts of functional tissue and plasticity are still present.

Sex hormones, too, are presumed to reactivate brain areas in aged rats and people, and in AD. In the aged rat, declines in AVP fiber density and in AVP-mRNA were observed, particularly in sex steroid-dependent areas (Goudsmit et al., 1988; Miller et al., 1989; Dobie et al., 1991) that coincided with the progressive age-related drop in plasma testosterone levels (Ravid et al., 1987; Goudsmit et al., 1990b). Testosterone supplementation was indeed shown to be able to restore the AVP innervation in old animals (Goudsmit et al., 1988, 1990c; Dobie et al., 1992). Furthermore, since testosterone is aromatized to estrogens in the brain, estrogens form a potentially important (re)stimulating factor as well. In view of the proposed decline in aromatase activity and decreased numbers and affinities of androgen receptors in senescent rat brain, estrogens or brain-specific estrogen receptor ligands may be even more effective than testosterone in stimulating sex-steroid dependent mechanisms in the senescent brain. In aged rats, the cessation of the estrus cycle in circulating estrogen results in suppression of hippocampal function, which could be restored by supplementing estradiol (Hagino, 1981). Other animal studies also show stimulatory effects of estrogens, but not of testosterone, on e.g. choline acetyltransferase activity in several brain areas as well as on memory and learning tasks (Goudsmit et al., 1990a,d; McBee et al., 1997).

Estrogens are presently frequently prescribed to postmenopausal women, as they have beneficial effects on several features of female aging, such as...
Bone loss (osteoporosis), hot flushes, nightly sweating, vaginal dryness and atrophy, heart disease and colon cancer as well as aging of the skin. Epidemiological evidence indicated that estrogen replacement therapy in postmenopausal women was effective in preventing and delaying the onset of AD (Henderson et al., 1996; Stephenson, 1996; Tang et al., 1996; Costa et al., 1999; Slooter et al., 1999; Van Duijn, 1999). Several studies have shown beneficial effects of estrogens on memory in postmenopausal women (Fedor-Freybergh, 1977; Sherwin, 1988; Phillips and Sherwin, 1992; Robinson et al., 1994; Haskell et al., 1997; LeBlanc et al., 2001), to enhance mental functioning in women with mild to moderate AD (Honjo et al., 1994; Ohkura et al., 1994a,b, 1995; Henderson, 1997), and to improve cognitive performance in patients with AD by enhancing the response to the acetylcholinesterase inhibitor tacrine (Schneider et al., 1996). In addition, improvements have been described in attention, memory, calculation, orientation and social interaction following administration of estrogens (Honjo et al., 1989). Estrogens significantly increase glucose metabolism in the lateral temporal region of non-demented elderly people (Rasgon et al., 2001). Postmenopausal estrogen replacement is furthermore considered to protect against AD (Henderson et al., 1996; Stephenson, 1996; Tang et al., 1996). However, a number of randomized controlled trials (RCTs) in AD patients could not show meaningful effects of estrogens (Henderson et al., 2000; Hogervorst et al., 2000; Mulnard et al., 2000; Wang et al., 2000; Yaffé et al., 2001). Other RCTs did show an enhanced cognition following estrogen treatment (Asthana et al., 1999, 2001; Carlson et al., 2001). There is a need for long-term RCTs with estrogens that start in postmenopausal mentally unaffected women.

Non-pharmacological stimuli

Older studies already showed that reality orientation — a long-term program of formal didactic group therapy — improved cognitive functioning of demented elderly people. This shows that the nature of staff attention in a nursing home is crucial (Woods, 1979; Hanley et al., 1981). A more recent study confirmed the positive effects of reality orientation on Mini Mental States Examination and verbal fluency scores (Zanetti et al., 1995). Also exercise therapy improved cognition in institutionalized geriatric mental patients (Powell, 1974). In two studies, elderly demented patients, including AD patients, received an ‘integrity-promoting care’ program consisting of increased emotional and intellectual communication and physical activation (Karlsson et al., 1985; Widerlöv et al., 1989). After applying this program for 2 months, short-term memory and visual perception had improved in the experimental group while they had deteriorated in the control group (Karlsson et al., 1985). Moreover, compared to the experimental group, concentration declined and absent-mindedness increased significantly in the control group. An important additional finding was that the experimental group showed an increase in their mean CSF level of the neuropeptide somatostatin, whereas the control group showed a decrease. In the other study, AD patients and patients with multi-infarct dementia (MID) received the ‘integrity-promoting care’ program for 3 months (Widerlöv et al., 1989). Short-term memory, dressing ability and physical activity improved, whereas confusion diminished. Moreover, the reduced CSF level of somatostatin had been elevated in the experimental group, whereas the concentration of vasopressin decreased in both groups, although to a lesser extent in the experimental group. A longitudinal study suggested that the engagement in leisure activities may reduce the risk of dementia (Scarmeas et al., 2001).

In a series of experiments, E.J.A. Scherder examined the effects of increased somatosensory input by means of various types of peripheral nerve stimulation (i.e. transcutaneous electrical nerve stimulation (TENS), tactile nerve stimulation, and a combination of both types of stimuli) on memory, and on independent and affective functioning of patients in a relatively early stages of AD (Scherder et al., 1992, 1995a,b,c, 1996, 1998; Table 1). In one study, the patients were treated 6 h/day, during a 6-week period (Scherder et al., 1992). In the other studies a 30-min/day treatment was applied during 6 weeks. Each treatment period was followed by a treatment-free period of 6 weeks. The results of these studies show that, compared to controls who received a placebo treatment, various aspects of non-verbal short-term memory, non-verbal and verbal long-term memory, and word fluency of stimulated AD patients im-
TABLE 1

Improvement of memory, independent, and affective functioning following transcutaneous nerve stimulation of the experimental group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Experimental group</th>
<th>Control group</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre Mean  SD</td>
<td>Pre Mean  SD</td>
<td>Post Mean  SD</td>
</tr>
<tr>
<td>Visual memory span</td>
<td>3.89  1.45</td>
<td>3.96  1.47</td>
<td>3.75  1.34</td>
</tr>
<tr>
<td>Eight words test recognition</td>
<td>5.58  4.54</td>
<td>7.50  3.66</td>
<td>5.00  4.11</td>
</tr>
<tr>
<td>Face recognition</td>
<td>4.14  3.73</td>
<td>4.17  4.63</td>
<td>3.67  3.70</td>
</tr>
<tr>
<td>Picture recognition</td>
<td>11.50  5.21</td>
<td>11.33  5.21</td>
<td>9.50  5.27</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>1.93  1.36</td>
<td>2.79  1.71</td>
<td>2.46  1.44</td>
</tr>
</tbody>
</table>

BOP subscales

| Need of help                 | 11.62  5.11        | 10.75  5.68   | 11.25  4.62   | 10.03  1.27   | 0.01 |
| Physical invalidity         | 1.58  0.83         | 1.38  0.92    | 1.75  0.89    | 12.17  1.27   | 0.01 |
| Inactivity                  | 7.58  2.41         | 7.88  3.14    | 8.25  2.76    | 4.61  1.27    | 0.05 |

Behavior inventory

| Overall affective behavior  | 10.46  11.12       | 0.88  10.99   | 4.88  1.28    | 0.04  |

Meta-analyses of the neuropsychological tests, BOP subscales and Behavior Inventory (overall affective behavior). The meta-analysis included data from five studies, i.e. Scherder et al., 1992, 1995a-c. BOP: Beoordelingsschaal voor Oudere Patiënten, a factor-analyzed rating scale for elderly patients. A lower score on a BOP subscale implies an improvement.

proved. More specifically, these improvements imply that, after treatment, patients were better capable of: (1) learning new material; (2) retrieving familiar, categorized information from their memory store; and (3) storing, reversing and reproducing non-verbal information (Table 1). With respect to independent and affective functioning, patients who were treated participated more independently in daily life, showed a better personal orientation and orientation in place, and enhanced their social interaction with fellow residents. In addition, stimulated patients felt less withdrawn, irritable, moody, dejected and gloomy and appeared to be more active and alert, possibly resulting in a decrease in forgetfulness. As in those studies, the therapist was present during both the peripheral stimulation of the experimental group and the sham stimulation of the control group, an effect of interpersonal communication could not be excluded. Consequently, it was examined whether TENS, in the absence of the therapist, could also have a positive influence on the cognitive, the independent, and the affective functioning of AD patients (Scherder et al., 1998). The results show that the improvements in non-verbal short- and long-term memory, verbal long-term memory and word fluency are solely due to the electrical stimulus itself. Furthermore, patients who were treated participated more independently in activities of daily life. However, TENS done in the absence of the therapist did not appear to have a beneficial effect on the patients’ affective functioning. Another finding was that the circadian rest–activity rhythm of stimulated AD patients improved (see also below), implying an increase in the strength of coupling to Zeitgebers, i.e. environmental cues (Van Someren et al., 1998). The results of the clinical studies further revealed that the majority of the effects of peripheral nerve stimulation, both in the presence and absence of the therapist, could not be maintained during the treatment-free period of 6 weeks.

In the above-mentioned studies, TENS was applied to patients in a relatively early stage of AD and the question arose whether TENS would exert similar positive effects in a more advanced stage of dementia. Consequently, TENS was applied to patients in a mid stage of AD (Scherder and Bouma, 1999). The results show that only the patients’ visual working memory significantly improved. Effects were found neither on other aspects of memory processes nor on (affective) behavior. It is noteworthy though that TENS appeared to have a beneficial effect on the rest–activity rhythm, similar to the one observed in patients in an early stage of the disease (Scherder et al., 1999). Despite the positive findings
of TENS in an advanced stage of AD, the effects are less than observed in an early stage of AD. It is concluded, therefore, that the effects of TENS may be stage-dependent.

As has been emphasized before, the ‘use it or lose it’ concept is not only applicable to AD but also to aging (Swaab, 1991). The results of a recent study show that TENS improved visual short-term and verbal long-term (recognition) memory, and semantic verbal fluency in non-demented elderly people (Scherder et al., 2000). Moreover, stimulated elderly subjects felt less depressed. An overall finding of the clinical studies was that, irrespective of the stage of dementia and presence/absence of the therapist, the majority of the effects could not be maintained during the treatment-free period of 6 weeks. We consider this finding to support a real treatment effect. After all, TENS or tactile stimulation are not expected to cure AD but, instead, to improve the quality of life of demented patients.

A related type of stimulation is transcranial electrostimulation (TCES or TES). In one study, TCES in elderly patients with multi-infarct dementia was also found to decrease wandering and nocturnal delirium and to enhance patients’ interaction with others (Hozumi et al., 1996). The authors suggest that the TES might partly be mediated through the somatic–sensory system. TES has recently been applied by us to patients in an early stage of AD. However, the results showed no improvement in cognition and (affective) behavior (Scherder et al., 2002). Further studies are required before firm conclusions on the (in)effectiveness of TES in AD can be drawn.

An age-related decrease in circadian modulation has, among other things, been observed in hormone levels, temperature, electroencephalographic (EEG) activity, alertness and sleep (Van Someren et al., 1993; Swaab, 1999; Van Someren, 2000a). Elderly people start napping during the day and often complain of disturbed sleep during the night (reviewed by Van Someren, 2000b). In AD, this fragmentation of the sleep–wake pattern is even more pronounced. The suprachiasmatic nucleus (SCN), which is the biological clock of the brain, is of critical importance in the circadian modulation of behavior and physiology. In aging, and even more so in AD, a marked reduction in the number of vasopressin-expressing neurons is found Fig. 9. The combined anatomical, physiological and behavioral findings suggest that a dysfunctional clock may underlie the sleep–wake pattern fragmentation (Swaab et al., 1985; Hofman and Swaab, 1994; Swaab, 1999; Liu et al., 2000), and we therefore tried a number of strategies designed to stimulate the circadian timing system in order to promote preservation of neuronal functioning of the circadian timing system, and thereby to enhance the functionality of the clock. Increased input to the circadian timing system can, among other things, be effectuated by means of bright environmental light, peripheral nerve stimulation and increased levels of physical activity. Our studies in aged rats have demonstrated improvement of both functional and anatomical signs of degeneration of the circadian timing system after environmental stimulation. Witting et al. (1993) demonstrated that the decreased amplitude in the circadian distribution of sleep and wakefulness as it is present in old rats, could be restored to the level of young rats by means of increasing the intensity of daytime environmental light. Lucassen et al. (1995) demonstrated that such increased light input counteracted the age-related decrease in the number of vasopressin-expressing neurons in the rat SCN.

In human, we have used the rest–activity rhythm as a marker of the functionality of the circadian timing system, because this variable can easily be assessed using actigraphy. An actigraph is a small wrist-worn solid-state recorder that continuously assesses the activity level, resulting in a time-series from which the strength of the circadian rhythm can be calculated. In a correlational study, we first investigated which constitutional and environmental factors were related to the severity of rhythm disturbances in AD patients. Regression analyses showed the most severe rest–activity rhythm disturbances in patients with a sedentary rather than physically active life style, and in patients exposed to low levels of environmental light (Van Someren et al., 1996). Subsequently, we investigated the effect of additional bright light on rest–activity rhythm disturbances in demented patients. Additional bright light improved the coupling of rest–activity rhythms to stable environmental cues (so-called Zeitgebers) in patients with intact vision, but not in patients with severely compromised sight (partial blindness, cataract) (Van Someren et al., 1997a). These results agree with
Fig. 9. Day–night fluctuation in vasopressin (AVP) mRNA of the suprachiasmatic nucleus (SCN) (expressed as masked area of silver grains) in controls and AD patients. Note that at any moment of the day, the values for AD patients are lower than those for controls. ▲, controls younger than 80 years; ●, controls older than 80 years; △, AD patients younger than 80 years; ◦, AD patients older than 80 years. The decreased amount of AVP expressed indicates low metabolic activity of the SCN in old people and even more so in AD patients. The diminished SCN metabolic rate in AD is held responsible for sleep disturbances and nightly restlessness. (From Liu et al., 2000.)

other studies showing improved circadian rhythms and decreased behavioral disorders in AD patients treated with bright light (Campbell et al., 1988; Okawa et al., 1989, 1991; Hozumi et al., 1990; Satlin et al., 1992; for further details see Van Someren et al., 2002, this volume). The observation that light therapy also increases the mini mental state scores in demented patients (Graf et al., 2001) makes light therapy of even greater interest for Alzheimer’s research. Recently it was shown that the age-related decrease in melatonin secretion, which is under the control of the SCN, is partly due to the poor illumination experienced by many elderly people and can be restored using bright light (Mishima et al., 2001).

The effect of additional physical activity in circadian rhythms was investigated in healthy elderly subjects, since fitness training was not a feasible option for most demented subjects. Fitness training improved the fragmentation of periods of rest and activity that occurs both during normal aging, and very pronounced after SCN lesions, as known from rat studies (Van Someren et al., 1997b). Whereas the effect of light and activity on the circadian timing system is well documented, the possible effect of somatosensory input to the SCN has only relatively recently been suggested by our group (Van Someren et al., 1998). In rats and squirrel monkeys, it has been demonstrated that the SCN is innervated by direct spinohypothalamic projections conveying somatosensory information (Cliffer et al., 1991; Newman et al., 1996). We have therefore investigated whether additional somatosensory input by means of TENS would provide an alternative means for the activation of SCN neurons. In early stage demented elderly people, repeated TENS was indeed found to improve the coupling of rest–activity rhythms to Zeitgebers, whereas placebo treatment was ineffective (Van Someren et al., 1998). Similar effects could also be obtained in advanced stages of AD (Scherder et al., 1999). The anatomical and functional findings from the reported studies indicate that the SCN retains considerable plasticity in old rats, and, in
functional terms, also in healthy and demented elderly subjects. In addition to the clinical relevance of manipulating circadian rhythms, the SCN appears to be a suitable structure for the study of the ‘use it or lose it’ concept (Swaab, 1991) as discussed in more detail by Van Someren (2002, this volume).

**Postmortem plasticity**

Animal models for human neurological and psychiatric diseases only partially mimic the underlying pathogenic processes. Therefore we investigated the potential use of cultures postmortem brain tissue from adult neurological patients and controls (Verwer et al., 2001). Organotypic human brain cultures obtained by autopsy within the framework of the Netherlands Brain Bank at 2–8 h after death can be maintained in vitro for extended periods and can be manipulated experimentally. Slices in basal medium supplemented with survival promoting neurotrophic factors retained more viable cells than slices in basal medium alone. Cytochrome oxidase activity could be enhanced by the addition of pyruvate as an extra energy source to the medium.

We also found, for the first time, that neurons in these cultures (motor cortex, hippocampus and cerebellum) could be transduced with adeno-associated viral vectors and expressed the reporter genes, EGFP (enhanced green fluorescent protein) and LacZ, for as long as 44 days, proving that the neurons were alive. These slice cultures offer new opportunities to study the cellular and molecular mechanisms of aging and neurodegenerative diseases. For instance, mutated genes involved in AD may be expressed in neurons of control patients to induce pathological alterations. Furthermore, putative therapeutic genes may be applied to brain slices of AD patients to enhance neuronal survival.

In conclusion, an increasing number of observations indicate that neuronal activation may slow down degenerative changes in aging and AD. An improved balance between DNA damage and repair could be the underlying mechanism, but alternative mechanisms are also possible. The beneficial effects of several types of neuronal activation may differ between different age groups or between different stages of AD. In general, activation seems to be more effective in younger age groups. Also, the APOE genotype and presence of functional reserves, whether or not induced by early exposure to a complex environment, profession or education may interfere with these effects. The effectiveness of neuronal stimulation within the physiological range may strongly depend on the use of the appropriate stimulus and whether the right receptors for certain stimulating factors are still present. Human neurons in tissue culture may be a model to test this. If these requirements are met, neuronal stimulation may indeed be effective in neuronal maintenance during aging and in AD and can thus be a fruitful basis for the search of a treatment strategy in AD.

The best way to prove that decreased metabolic activity indeed plays a major role in the development of dementia, is of course to show that reversing decreased neuronal metabolism would lead to considerable improvement of cognitive functions. The first series of data offer proof of the principle that such a strategy may be effective, and the observations that glucose administration or increasing glucose availability by hyperinsulinemia enhances memory in patients with probable AD (Manning et al., 1993; Craft et al., 1996) not only support the view that AD is basically a hypometabolic disease, but also indicate that the focus on metabolic stimulation of neurons seems to be a fruitful strategy.

**Summary**

(1) Alzheimer’s disease is a multifactorial disease in which age and APOE-ε4 are important risk factors.

(2) The neuropathological hallmarks of AD, i.e. amorphous plaques, neuritic plaques (NPs), pretangles, neurofibrillary tangles (NFT) and cell death are not part of a single pathogenetic cascade but may occur independently.

(3) In brain areas where classical AD changes, i.e. NPs and NFTs, are present, such as the CA1 area of the hippocampus, the nucleus basalis of Meynert and the tuberomamillary nucleus, a decreased metabolic rate is found. The decreased metabolic rate appears not to be induced by the presence of pretangles, NFT or NPs.

(4) Decreased metabolic rate may precede cognitive impairment and is thus an early occurring hallmark of AD, which, in principle, may be
reversible. The observation that the administration of glucose or insulin enhances memory in AD patients also supports the view that AD has a metabolic basis.

(5) Moreover, several observations in postmortem brain indicate that activated neurons are better able to withstand aging and AD, a phenomenon paraphrased by us as ‘use it or lose it’.

(6) It is, therefore, attractive to direct the development of therapeutic strategies towards restimulation of neuronal metabolic rate in order to improve cognition and other symptoms in AD. A number of pharmacological and non-pharmacological studies support the concept that activation of the brain has beneficial effects and may, to a certain degree, restore several aspects of cognition and other central functions. For instance, the circadian system may be restimulated in AD patients by exposing them to more light or transcutaneous nerve stimulation. A procedure has been developed to culture human postmortem brain tissue that allows testing of the efficacy of putative stimulatory compounds such as neurotrophins.

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