Reduced Neuronal Activity is One of the Major Hallmarks of Alzheimer's Disease

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Introduction

Alzheimer's disease (AD) is histopathologically characterized by the presence of neuritic plaques (NPs) and cytoskeletal changes (Fig. 1) that are visible as pretangles stained by Alz-50, neurofibrillary tangles (NFT) in the cell bodies of affected neurons, and neuropil threads (Braak et al. 1986) or dystrophic neurites (Kowall and Kosik 1987). Dystrophic neurites are defined as short, thickened, curly, coiled or sometimes hooked fibres observable as the neuritic component of NPs or present in the neuropil outside these structures. Neuropil threads is an alternative term for dystrophic neurites that are not the neuritic component of NPs. To a lesser degree, NPs and cytoskeletal changes can also be observed in aged, nondemented control subjects.

This review will provide evidence in favour of the thesis that 1) the neuropathological Alzheimer changes cannot all be explained by a cascade starting with amyloid deposits as suggested, e.g., by Selkoe (1994), 2) the neuropathological hallmarks of AD are basically independent phenomena and 3) neuronal inactivity is one of the major characteristics of AD and may underlie the clinical symptoms of dementia.

Amyloid: Just one of the Many Factors Involved

The amyloid cascade hypothesis, as advocated by Selkoe (1994), constitutes a major working hypothesis in current AD research. Amorphous plaque deposition is thought to be the primary event. The congophilic components of NPs are assumed to arise from the congo-negative amorphous plaques by aggregation of β /A4-protein fibrils. Due to the neurotoxicity of β /A4, NP formation, the occurrence of neuropil threads and dystrophic neurites would be induced followed by NFT formation and ultimately cell death. The toxic potency of amorphous plaque deposits is attributed to the β /A4-protein (Masters et al. 1985) that consists of 40–43 amino acids that are cleaved from the much larger amyloid precursor protein (APP; Kang et al. 1987). Grafting genetically transformed cells that over-

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- ◀ **Fig. 1.** Localization of cytoskeletal changes and amorphous plaques. A. Alzheimer's disease (AD) patient, 90 years of age. A4-reactive plaque deposits (ap) and Alz-50-stained dystrophic neurites (d) and perikarya (p) are found close together in the seriously affected AD frontal cortex. The A4-antiserum used for this staining was SP28. B. AD patient, 40 years of age. The occurrence of amorphous plaques (ap) and dystrophic neurites (d) is more discrete in the AD hypothalamus than in the AD cerebral cortex. Although occasionally Alz-50 positive dystrophic neurites can be found apposed to amorphous plaques (*), cytoskeletal changes are mostly not locally accompanied by plaque deposits. C. AD patient, 70 years of age. The nucleus tuberalis lateralis (NTL) filled with Alz-50-stained cytoskeletal changes can easily be distinguished from the tuberomammillary nucleus (TMN), which shows a considerably lower density of AD-related altered cytoskeletal structures. Note that the vast majority of cytoskeletal changes are not accompanied by A4-reactive plaque deposits (ap). The A4 antiserum used was SP28. D. AD patient, 40 years of age. Alz-50-stained dystrophic neurites (d) in the suprachiasmatic nucleus (OC, optic chiasm) were never observed to coexist with SP28-reactive plaque deposits (arrowheads indicate the borders of the suprachiasmatic nucleus). All bars represent 100 μm , except that in Fig. 1B, which represents 50 μm (from Van de Nes et al. 1994, with permission).

express β /A4 amyloid into the suprachiasmatic nucleus of adult rats indeed caused a disruption of circadian rhythms, a finding that favours the idea that this compound is neurotoxic (Tate et al. 1992). Moreover, AD is linked to trisomy 21 (Down syndrome) and in some cases to a point mutation in the APP gene on chromosome 21. Although there are certainly arguments in favour of Selkoe's cascade hypothesis, there are also data from a large number of papers that do not fit into this hypothesis (for review see Van de Nes et al. 1994). The vast majority of AD cases (99.5 %) are not linked to APP gene mutations or an extra copy of chromosome 21 (Rossor 1993). The occurrence of familial AD cases linked to chromosomes other than chromosome 21, e.g., chromosome 14 (Mullan et al. 1992; Schellenberg et al. 1992; St George-Hyslop et al. 1992; Van Broeckhoven et al. 1992), or 1 (Levy-Lahad et al. 1995) as well as the cases linked to chromosome 19 (Pericak-Vance et al. 1991; Strittmatter et al. 1993a, b) cannot be explained in a direct way by the amyloid cascade hypothesis.

Cytoskeletal Changes without Senile Plaques

The histopathological changes in the punch-drunk dementia syndrome of boxers (dementia pugilistica) also do not fully agree with the amyloid cascade hypothesis. The hippocampus and cortex of AD-demented boxers show many amorphous plaques, NFTs and neuropil threads, but very few if any NPs (Roberts et al. 1990). In several cases of Alzheimer patients, Bouras et al. (1994) found NFTs without amyloid deposition. These observations indicate that cytoskeletal changes may occur independent of aggregated β /A4 fibril amyloid cores.

No Transformation of Amorphous to Neuritic Plaques (NPs)

It is presumed by many authors that there is a transformation from amorphous plaques to NPs, but the relationship between these AD changes is equivocal both in histopathological and biochemical terms. Amorphous plaques do not seem to be neurotoxic and induce NFT. Cytoskeletal changes and amorphous plaques are not related in their localization (Fig. 1). In the hippocampus and neocortex of aged, nondemented control subjects, large amounts of amorphous plaques may be present, whereas NFTs are practically absent (Duyckaerts et al. 1988; Barcikowska et al. 1989; Delaere et al. 1990) or the presence of neuropil threads is low (Dickson et al. 1988; McKee et al. 1991). Amorphous plaques are present in the cerebellum of AD and Down syndrome patients without the presence of neurofibrillary changes in the brain area (Braak et al. 1989a; Joachim et al. 1989). In addition, the widespread presence of amorphous plaques is not necessarily associated with AD dementia. The percentage of aged, nondemented subjects over 75 years of age with β /A4 deposits in the cortex may reach 80 % (Davies et al. 1988), whereas for the parahippocampal and superior temporal gyri of nondemented centenarians this proportion may even approximate 100 % (Delaere et al. 1993). In addition, large amounts of amorphous plaques may also be found with hardly any neurofibrillary changes in the parvocellular layer of the presubiculum (Kalus et al. 1989; Akiyama et al. 1990). Also, Bouras et al. (1994) found that the extent of amyloid deposition in the hippocampal area was not correlated with the diagnosis of Alzheimer's disease.

Amorphous plaque deposition in the brain is rather diffuse, whereas SPs are concentrated in particular areas. Amorphous plaques are found in all six neocortical layers (Braak et al. 1989b; Arnold et al. 1991), whereas classical and NPs are mainly found in layers II–III (Pearson et al. 1985; Rogers and Morrison 1985; Duyckaerts et al. 1986).

Furthermore, amorphous plaques also occur in 66 % of the elderly (i.e., over 65 years of age) affected by neurodegenerative disorders other than AD, e.g., progressive supranuclear palsy, Parkinson's disease, Huntington's chorea and frontal lobe dementia. However, amorphous plaques are widely associated with NPs and neuropil threads in AD only (Mann and Jones 1990).

The transformation from amorphous plaques into NPs can also be questioned on the basis of the finding that amorphous plaques stain with antibodies raised against the β /A4-protein and the APP extracellular epitope, but not with antisera raised against the APP carboxy-terminal region, whereas classical NPs stain with all three antisera (Tagliavini et al. 1991). These observations indicate that there may also be differences in the chemical composition of classical NPs and amorphous plaques in addition to a difference in the aggregated versus the nonaggregated state of the β /A4-protein fibrils.

The appearance of plaques that might represent intermediate forms between amyloid plaques and NPs has never been reported.

Neuritic Plaques and Neurofibrillary Tangles may Occur Independently

The NPs with a congophilic amyloid core have obtained particular attention, since amyloid has a central place in the cascade hypothesis. However, it is not the NPs but the cytoskeletal changes that parallel the duration and severity of AD (Arriagada et al. 1992; Braak and Braak 1991; McKee et al. 1991; Mukaetova-Ladinska et al. 1993). NFTs are often proposed to have their starting point in the neurotoxicity of NPs. This idea is supported by the observation that many classical NPs are present in the "terminal zone of the perforant pathway," which corresponds to the outer two-thirds of the molecular layer of the dentate gyrus and to the distal dendrites of the subicular CA1 zone (Hyman et al. 1986). The neurons of the perforant pathway projections that originate from the entorhinal cortex in fact contain many NFTs in AD (Hyman et al. 1986). However, the idea that the formation of NFTs in entorhinal cortex neurons and of NPs in the hippocampal CA1 region is necessarily linked is not supported by statistical analysis. The correlation between the density of cellular NFTs in the entorhinal cortex neurons and classical NPs in the dentate gyrus and CA1 region as projection area appears to be weak (Armstrong et al. 1992). In addition, clusters of NPs and tangles are spatially not related (Armstrong et al. 1993). Moreover, 30 % of the demented senile "Alzheimer patients" with plaques in the neocortex appeared to lack tangles in this brain area (Terry et al. 1987). This finding suggests that the occurrence of classical SPs on the one hand and NFT formation on the other is rather independent.

There is also no precise relationship between the clustering of NPs in the neocortex on the one hand and the localization of the terminals of the magnocellular cholinergic neurons of the basal forebrain or noradrenergic cells in the locus coeruleus that contain the NFT. In the neocortex, NPs predominantly occur in layers II–III (Pearson et al. 1985; Rogers and Morrison 1985; Duyckaerts et al. 1986), whereas data on cholinesterase histochemistry show the most dense staining in layers I and VI (Mesulam et al. 1983). These are precisely the neocortical laminae that have the fewest NPs. This means that the layers to which the magnocellular cells of nucleus basalis of Meynert (NBM) project do not coincide with the layers with the densest NP concentration. In addition, dopamine- β -hydroxylase histochemistry shows that the locus coeruleus neurons also mainly send their axons to layers I and VI (Morrison et al. 1982). Thus, the neocortical layers to which locus coeruleus neurons send their axons do not correspond with the neocortical layers that show a concentration of NPs, i.e., layers II and III. Therefore, NFT formation in the cholinergic neurons of the NBM and the noradrenergic cells of the locus coeruleus appears to be independent of NP deposition in the neocortex.

The possibility of independent formation of NPs and NFT is further supported by the reported cases of AD that show a lack of NFTs (Terry et al. 1987).

Similar discrepancies are found in the striatum. AD-affected type IV and V interneurons in the caudate nucleus and putamen develop NFTs and dystrophic

neurites, but hardly any classical NPs are found (Braak and Braak 1990). This indicates that NFT formation in the striatum is a local pathology not induced by classical NP deposition in this structure.

Another histopathologic argument that suggests that cytoskeletal changes are not necessarily locally induced by aggregated β /A4-protein fibrils is provided by our observations in the hypothalamus. Firstly, congophilic plaques in the hypothalamus are rare (Standaert et al. 1991). However, various hypothalamic nuclei appear to be affected by Alz-50-stained "pretangle" cytoskeletal changes (Swaab et al. 1992; Van de Nes et al. 1993). In addition, the hypothalamic nucleus tuberalis lateralis (NTL) undergoes hardly any amorphous or NP formation in AD (Kremer et al. 1991; Van de Nes et al. 1996), although the nucleus stains intensively with Alz-50-stained cytoskeletal changes (Kremer et al. 1991; Swaab et al. 1992; Van de Nes et al. 1993; see Fig. 1). The hypothalamic suprachiasmatic nucleus may show some Alz-50-stained cell bodies and altered fibres in AD, but amorphous or classical plaques have never been observed in this nucleus (Fig. 1).

These observations suggest that induction of pretangles, neuropil threads and NFTs by neurotoxic NPs does not seem to play a crucial role in AD development, as proposed in the amyloid cascade hypothesis. Rather, NP formation has to be seen as an independent process. This would also explain why no spatial relationship is seen between the distribution of neuropil threads and NPs. In addition, it would clarify why large amounts of NPs can be found in the neocortex of nondemented patients, whereas neuropil threads (Dickson et al. 1988; McKee et al. 1991) or NFTs are virtually absent (Katzman et al. 1987; Crystal et al. 1988; Arriagada et al. 1992).

Cytoskeletal Changes and NPs do not Induce Cell Death

The last step of the amyloid cascade hypothesis is the idea that neurons producing cytoskeletal changes are indicative of impending neuronal death and that cell death would be induced by the neurotoxic NPs.

The findings in the AD locus coeruleus are in agreement with this last part of the hypothesis. Large neuromelanin pigment-containing neurons in the locus coeruleus develop NFTs, and neuronal death may be up to 80 % (Bondareff et al. 1982; Chan-Palay and Asan 1989; German et al. 1992; Hoogendijk et al. 1995). The proposed sequence, i.e., that NFT formation is followed by dramatic cell loss, was thought to be common AD. However, this only holds for a few brain areas (e.g., locus coeruleus and CA1 of the hippocampus). Cell loss is indeed considerable in the latter structure in AD (West et al. 1994). This is in contrast to the NBM, in spite of the fact that in early reports, neuronal loss of up to 90 % has also been reported (Mann et al. 1984; Whitehouse et al. 1982), only a small part of which was related to aging (Lowes-Hummel et al. 1989; De Lacalle et al. 1991). However, the reported loss in the large-sized cholinergic neurons in the NBM in AD appears to be related to cell shrinkage, reduced activity, and loss of cholinergic cell markers rather than to cell loss (Rinne et al. 1987; Allen et al. 1988;

Salehi et al. 1994). Regeur et al. (1994), using unbiased sampling and counting methods, showed that global neocortical cell loss does not take place in this brain area of AD patients, providing strong evidence that neuronal shrinkage rather than cell death is a major phenomenon in AD.

Data on Alz-50-stained cytoskeletal changes and neuronal cell counts of the sexually dimorphic nucleus (SDN) and NTL in control and AD hypothalami of different ages do not support the idea that neurons staining for cytoskeletal changes are indicative of impending cell death. Alz-50-stained cell bodies and dystrophic neurites in the SDN have only been observed in AD and not in controls (Swaab et al. 1992; Van de Nes et al. 1993), whereas the pattern of loss of SDN neurons in AD is similar to that during normal aging (Swaab and Hofman 1988). In addition, the Alz-50 staining of the NTL of AD patients is so abundant that it can even be seen with the unaided eye, although neuron number in AD is not different from that in control subjects (Kremer et al. 1991).

The CA1 area of the hippocampus is one of the few areas that shows a very clear neuronal loss in AD. West et al. (1994) have shown that the number of neurons in the CA1 area of the hippocampus is much more reduced in AD than in normal aging. Since this area shows not only a massive number of NFTs but also NPs in AD, the CA1 was chosen to study the question of whether or not SPs may induce local cell death (Emre et al. 1992; Kowall et al. 1992). Our study (Salehi et al., submitted for publication) shows 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 this effect was dose dependent. However, it should be noted that the contribution of this effect on the total cell death in the CA1 area is very limited, i.e., 2,6 %. This study again supports the notion that SPs and cell death are largely two independent phenomena.

No Animal Models for AD

Based on observations on mutations in the APP gene in some familial AD cases and early-onset AD dementia in trisomy 21 Down syndrome patients that is presumed to be related to overproduction of normal APP, various transgenic mouse models overexpressing APP have been developed and tested. However, the results obtained thus far are rather disappointing. A few years ago, a major step forward was claimed by Kawabata et al. (1991), who reported the detection of amyloid deposits and NFTs in the transgenic mouse brain. However, the authors had to retract their paper since they could not reproduce the histopathological AD changes. Another research group reported the presence of small deposits of age-related β /A4-protein-like immunoreactivity in the mouse hippocampus (Wirak et al. 1991). These deposits, however, appeared to be clusters of intracytoplasmic inclusions in astrocytic processes, i.e., a murine corpora amylacea-like structure that can exhibit nonspecific staining patterns with a variety of polyclonals (Jucker et al. 1992). Other transgenic mouse models with an overproduction of

normal APP revealed some β /A4-amyloid-like deposits in the hippocampus and cortex, but significant behavioral changes or additional histopathological changes associated with AD were not found (Quon et al. 1991; Kammesheidt et al. 1992; Andr et al. 1996; Games et al. 1995; Malherbe et al. 1996). Even the best animal model, described by Games' group (Greenberg et al. 1996) using an APP mutation that gives a 10-fold increase in the level of APP, did not show NFTs (Games et al. 1995). In addition, human neurons that secrete A β did not induce Alzheimer pathology following transplantation into rodent brain (Mantione et al. 1995). Thus, so far, no animal model has yet been developed that can support the amyloid cascade hypothesis.

Decreased Neuronal activity is a Major Hallmark of Alzheimer's Disease

It has been proposed that decreased neuronal activity is an essential characteristic of AD and that neuronal activity would protect against the degenerative changes of aging and AD, an hypothesis paraphrased as "use it or lose it" (Swaab 1991). In fact, there are studies in the literature supporting the view that decreased metabolic rate is a major hallmark of AD. It has been reported that the AD brain shows a lower total amount of protein (Suzuki et al. 1965), a clear reduction in total cytoplasmic (Bowen et al. 1977; Mann et al. 1981; Doeblner et al. 1988) and messenger RNA (Sajdel-Sulkowska and Marotta 1994; Guillemette et al. 1986; Taylor et al. 1986), reduced glucose metabolism especially in temporal and parietal lobes, as shown by positron emission tomography (Hoyer et al. 1988; Kumar et al. 1993; Meneilly and Hill 1993; Meier-Ruge et al. 1994; Swerdlow et al. 1994), a smaller size of the neuronal Golgi apparatus (Salehi et al. 1994, 1995b, c) and a lower cytochrome oxidase level (Simonian and Hyman 1993, 1994). In this respect it is also interesting to note that isolated microvessels from Alzheimer patients' temporal cortex showed decreased glucose metabolism, suggesting a global defect in brain energy metabolism (Marcus et al. 1989).

Foster et al. (1984) have demonstrated that a substantial decrease in cerebral glucose metabolism may precede significant cognitive impairment. This observation was supported by Reiman et al. (1996), who found that late middle-aged, cognitively normal subjects who are homozygous for the APOE E4 allele, and thus at risk for AD, have already reduced glucose metabolism in the same region of the brain as is later affected in patients with probable AD. Reduction of regional cerebral glucose metabolism in later stages of AD is related to neuropsychological impairment (Haxby et al. 1988; Mielke et al. 1994). It is interesting to note that Parkinson patients with dementia also show a global decrease in glucose metabolism similar to that in AD, i.e., with more severe abnormalities in the temporo-parietal region (Peppard et al. 1992). It is presumed that cortical glucose hypometabolism in AD may reflect reduced synaptic activity (Salmon et al. 1996).

Two major questions concerning the pathogenesis of AD were therefore 1) whether the presence of plaques and tangles in AD was indeed related to decreased neuronal activity in the various brain areas, and, if so, 2) whether these neuropathological AD hallmarks would induce decreased metabolic rate or vice versa, or alternatively whether the neuropathological AD changes and decreased metabolic rate would occur independently. Our recent research supports the latter possibility.

Relationship Between Alzheimer Neuropathology and Decreased Metabolism

Using the size of the Golgi apparatus as a histological parameter of chronic activity changes in neurons, we studied the relationship between the activity of neurons in areas with different types of neuropathological AD changes. The hypothalamus contains several nuclei that are differentially affected in AD. For instance, the supraoptic nucleus (SON) is not affected at all by AD changes and even shows hyperactivation during aging, both in controls and AD patients (Swaab et al. 1992; Lucassen et al. 1994). This is in contrast to the NBM, which shows clear signs of atrophy (Rinne et al. 1987), cytoskeletal alterations (Swaab et al. 1992; Van de Nes et al. 1993) and some NP formation (Rudelli et al. 1984). In addition, we studied the CA1 area of the hippocampus, a brain region that is affected by cell death (West et al. 1994), an abundance of NFTs and a moderate amount of NPs (Mann et al. 1985). In the hypothalamic and hippocampal tissue from controls and AD patients, we investigated the possible presence or absence of a relationship between different stages of neurofibrillary degeneration and neuronal activity and wondered what could be cause or effect of such a relationship. In order to do so, the relationship between Golgi apparatus size and the following stages of AD changes were assessed in case of:

No AD changes

The SON of the hypothalamus appears to be completely spared in AD. No classical AD neuropathology is present and, even using antibody Alz-50 as an indicator of early cytoskeletal alterations (Baner et al. 1989), no staining of SON neurons is found (Swaab et al. 1992; Van de Nes et al. 1993). Furthermore, no cell loss in the SON is found either in aging or AD (Goudsmit et al. 1990; Van der Woude et al. 1995). In contrast, even signs of hyperactivation with aging were found in the SON (Hoogendijk et al. 1995; Van der Woude et al. 1995). As shown by Lucassen et al. (1994; Fig. 2), there was indeed a significant increase in activity of vasopressinergic neurons of the SON during aging, both in controls and AD patients, supporting the idea that activation of neurons might protect them against AD changes (Swaab 1991).

Fig. 2. Photomicrograph depicting Golgi apparatus (MG-160) staining in vasopressinergic SON neurons of the hypothalamus of a 43-years-old control subject (A), an 82-year-old control subject (B), a 49-year-old Alzheimer's patient (C) and an 81-year-old Alzheimer's patient (D). Note the increase in size of the Golgi apparatus with age. Bar represents 28 μ m (from Lucassen et al. 1994, with permission).

Early Cytoskeletal Alterations

Tau proteins belong to the microtubule associate proteins. Abnormal phosphorylation of tau proteins as found in AD patients may lead to failure of tau proteins to bind to microtubules (Lee et al. 1991). This would result in a failure to maintain axonal transport and neuronal shape. It has been suggested that the occurrence of early cytoskeletal changes due to abnormal phosphorylation of tau, as found by a variety of antibodies, would precede the appearance of neurofibrillary degeneration, as shown by silver staining (Baner et al. 1989; Braak et al. 1994).

The hypothalamic nucleus tuberalis lateralis (NTL) shows strong cytoskeletal alterations, as appears from the intense staining of NTL neurons by the antibodies Alz-50, tau-1 (against tau) and 3-39 (against ubiquitin; Swaab et al. 1992; Van de Nes et al. 1993; Fig. 1). Interestingly, silver stained NFTs and NPs are rare in the NTL of AD brains (cf., Kremer 1992). The NTL thus represents a brain area that shows an early stage of AD changes and does not progress towards classical silver staining of neuropathological AD hallmarks (Swaab et al. 1992), which made it a very suitable structure for a study of the relationship between the presence of pretangles and changes in neuronal activity. As shown in Salehi et al. (1995a), there was no reduction in the activity of this area in AD (Fig. 3). Furthermore, comparison of the intensity of Alz-50 staining with Golgi apparatus (GA) size did not show a clear relationship between these two parameters. This study showed that strong cytoskeletal alterations in the NTL are not accompanied by decreased neuronal activity and that pretangle AD changes and reduced metabolic activity are not necessarily related.

Late Cytoskeletal Alterations (NFTs)

The appearance of early cytoskeletal alterations is presumed to be followed by the formation of NFTs that are detectable by silver staining (Baner et al. 1989). The finding (see above) that there was no relationship between the appearance of early cytoskeletal alterations and protein synthetic ability in the NTL raised the question whether AD changes and decreased metabolism would be related in areas with late stages of cytoskeletal alterations, i.e., NFTs and SPs such as the NBM, tuberomammillary nucleus (TM) and CA1 area of the hippocampus.

The NBM is an area of the basal forebrain that is severely affected in AD. This nucleus shows not only early cytoskeletal alterations, as indicated by Alz-50 staining, but also NFTs and β -amyloid accumulation and NPs in AD (Rudelli et al. 1984; Swaab et al. 1992; Van de Nes et al. 1993). Although it was suggested initially that this area shows dramatic cell death in AD (Whitehouse et al. 1982; 1983), it turned out that degeneration in the NBM is characterized by cell atrophy rather than by cell death (Pearson et al. 1983; Rinne et al. 1987). A significantly decreased size of the GA was found in NBM neurons in AD, suggesting that protein synthetic activity of NBM neurons is strongly reduced in this brain area (Salehi et al. 1994; Fig. 4).

Fig. 3. Immunocytochemical staining of the Golgi apparatus (GA) in the nucleus tuberalis lateralis of a young control (A) and old control (B) and young AD patient (C) and old AD patient (D). Note the similarity between the GA of cells of the four groups. Scale bar 30 μ m (from Salehi et al. 1995a, with permission).

Fig. 4. Immunocytochemical staining of the Golgi apparatus (GA) in the nucleus basalis of Meynert of a young (A) and old (B) control and young and old AD patients (C, D). Note the clear reduction in size of the GA in AD patients when compared to the controls (from Salehi et al. 1994, with permission).

As shown by Salehi et al. (1995c), metabolic activity of TM neurons, an area of the hypothalamus that is clearly affected by NFTs (Nakamura et al. 1993), is also significantly reduced in AD, which supported again the existence of a relationship between AD pathology and decreased neuronal activity. The same holds for the CA1 area of the hippocampus that is strongly affected by AD changes (West et al. 1994) and where, as shown by Salehi et al. (1995b), neuronal activity was strongly decreased in AD patients.

In conclusion, the pretangle stage of AD changes is not necessarily related to changes in metabolic rate as indicated by our studies in the NTL. However, both 1) the clear reduction in Golgi apparatus size in the AD affected NBM, TM and CA1 neurons and 2) the significant increase in activity of the vasopressinergic neurons during aging and AD in the SON, an area where no cytoskeletal abnormalities are found, support the idea that decreased neuronal activity and the occurrence of the classical AD changes go together. Yet, these observations could not reveal the causality of such a relationship.

Tangles and Neuritic Plaques (NPs) do not Cause Decreased Metabolic Rate

The next step was to study the causality of the relationship between the presence of NPs and NFT in a brain area with decreased metabolic activity. For this purpose we compared the metabolic rate 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. The presence of NFT does not seem to decrease the metabolic rate of a neuron (Salehi et al. 1995b). So, although NFT and decreased metabolic activity are present in the same brain area, i.e., CA1, they do not seem to be causally related. This finding agrees with the observation of Gertz et al. (1989), who showed that the presence of intraneuronal NFT in the CA1 area of the hippocampus is not related to other parameters of metabolic activity, i.e., nucleolar or cell size.

NPs are considered by some to be later stages of amorphous plaques (Roze-muller et al. 1989). Because of extensive damage to the neuropil in the area 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 and induces neural degeneration. On the other hand, unlike 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 questionable. If a plaque contained 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 importance of such a mechanism. There appeared to be no relationship between either the density of NPs or the distance of each NP to the activity of neighbouring neurons (Salehi et al., submitted for publication). This finding does not support the possibility that neurotoxicity of plaques caused decreased neuronal metabolism but rather that metabolism and NPs are two basically independent phenomena.

Restoration of Neuronal Activity

The present review showed that there is a clear reduction in neuronal metabolic activity in various brain areas in AD patients. On the basis of this important hallmark of AD (Swaab 1991), one may assume that restoration of the activity of neurons would lead to diminishment of cognitive impairment. Although it is not yet clear whether decreased metabolic activity is a primary process in the course of AD, it has been shown that, in principle, reactivation of neurons might be beneficial for AD patients. Global stimulation by exercise, transcutaneous electric nerve stimulation (TENS), tactile nerve stimulation and a combination of the latter two significantly improved memory and affective behaviour of patients with probable AD (Powell 1974; Scherder et al. 1995a–c). Fragmentation of sleep-wake patterns occurs in senescence and even more clearly in AD (Witting et al. 1990), which is believed to be due to degenerative changes in the suprachiasmatic nucleus (SCN; Swaab et al. 1985, 1992). The SCN receives a strong input from the retina. It has been shown that exposure of AD patients to more intense light improves sleep-wake rhythms in clinical trials of these patients (Hozumi et al. 1990; Okawa et al. 1991; Mishima et al. 1994; Satlin et al. 1992). These clinical observations agree with the experimental data showing that degenerative changes in the SCN of aged rats, such as decreased amplitude of sleep-wake parameters and decreased numbers of vasopressin-expressing neurons in the SCN, could be counteracted by stimulation of the SCN by light (Witting et al. 1993; Lucassen et al. 1995).

The best way to prove that decreased metabolic activity indeed plays a major role in the development of dementia is of course to show in the future that reversing decreased neuronal activity would lead to considerable improvement of cognitive functions. The observation that glucose administration enhances memory in patients with probable AD (Manning et al. 1993) indicates that the focus on metabolic stimulation of neurons might be a fruitful strategy.

Summary and Conclusions

1. The neuropathological hallmarks of AD, i.e. amorphous plaques, NPs, pretangles, NFT and cell death are not part of a pathogenetic cascade but are basically independent phenomena.
2. Pretangles can occur in neurons in which the metabolic rate is not altered. However, in brain areas where classical AD changes, i.e., NPs and NFT, are present, such as the CA1 area of the hippocampus, the nucleus basalis of Meynert and the tuberomammillary nucleus, a decreased metabolic rate is found.
3. Decreased metabolic rate also seems to be an independent phenomenon in AD. It is not induced by the presence of pretangles, NFT or NPs.
4. Decreased metabolic rate is considered to be crucial and an early hallmark of AD. In theory, decreased metabolic rate may be reversible. It is, therefore,

attractive to direct the development of therapeutic strategies towards restimulation of neuronal metabolic rate to improve cognition and other symptoms in AD.

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