NEURODEGENERATION AND THE CHOLINERGIC SYSTEM

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Neurodegeneration is a feature of several chronic brain diseases which for this reason are defined neurodegenerative. Some of them are characterized by the prevalent, but not exclusive, degeneration of a type of neuron. Examples are the degeneration of the cholinergic neurons in Alzheimer's disease and dopaminergic neurons in Parkinson's disease. This review deals with neurodegenerative diseases in which a relevant loss of cholinergic neurons has been shown, including Alzheimer's disease, Parkinson's and parkinsonian diseases, and alcoholic dementia . Aims of the review are 1) to describe the alterations of the cholinergic neurons leading to their death and cholinergic denervation of some brain areas, 2) to discuss the mechanisms responsible for the loss of the cholinergic neurons, and 3) to evaluate the role of their degeneration in the clinical features of the diseases. On the basis of the existing data, it may be assumed that neurodegeneration of the cholinergic neurons in Alzheimer's disease is caused by the β-amyloid overload which exerts a direct toxic effect through p75(NTR) receptors and an indirect effect through an inflammatory reaction. The products of neuroinflammation reduce the availability of NGF, needed for the cholinergic neurons survival, and increase the level of pro-NGF which is toxic for the cholinergic neurons. In Parkinson and parkinsonian diseases, alpha-synuclein toxicity may be responsible for the degeneration of the midbrain cholinergic neurons and contribute to that of the forebrain neurons. Finally, much evidence indicate that the loss of forebrain cholinergic neurons is largely responsible for the cognitive deficits of dementias.

The term neurodegeneration refers to a progressive process of neuronal, myelin or tissue breakdown resulting in changes in the morphology and function of neurons usually leading to their death. The damage and death of the neurons is associated with an inflammatory response (Wiss-Coray and Mucke, 2002) which involves an extensive glia activation and plays a role in the neurodegenerative process (Varnum and Ikezu, 2012). Neurodegeneration characterizes several brain diseases which for this reason are defined neurodegenerative. The most important are Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis, but also alcohol abuse and trauma may lead to neurodegeneration. Neurodegeneration followed by neuron death can be induced in experimental animals in discrete brain regions by the injection of neurotoxins (Olton and Wenk, 1987) and by inducing a neuroinflammatory response through intracerebroventricular infusion of bacterial lipopolysaccharides (Willard et al, 1999). Among the neurotoxins, ethylcholine mustard aziridinium (AF64A), an analog of choline which inhibits irreversibly high affinity choline uptake, has been shown to have some selectivity for the cholinergic neurons (Mantione et al, 1981). A

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selective degeneration of the forebrain cholinergic neurons can be obtained by local injection of the immunotoxin 192 IgF saporin (Wiley et al, 1991; Ballmayer et al, 2001). The immunotoxin acts by coupling the ribosome inactivating toxin saporin to an antibody that recognizes low-affinity nerve growth factor (NGF) receptors, which are found in cholinergic neurons of the basal forebrain.

The neurodegeneration may be diffused throughout the brain involving neurons and glial cells. However, some neurodegenerative diseases are characterized by the prevalent, but not necessarily exclusive, degeneration of a type of neuron. Examples are the degeneration of cholinergic neurons in AD (Whitehouse et al, 1982) and dopaminergic neurons in PD (Hornykiewicz, 1971).

Aims of this review are: 1) to describe which cholinergic neurons degenerate, with the ensuing cholinergic denervation of some brain areas, in some neurodegenerative disease, namely AD, PD, Parkinsonian diseases and alcoholic dementia; 2) to discuss the mechanisms responsible for the degeneration of the cholinergic neurons, and 3) to evaluate the role of the loss of the cholinergic neurons in the clinical features of the diseases. The changes in the nicotinic and muscarinic receptors associated with the cholinergic denervation are beyond the scope of this review.

THE BRAIN CHOLINERGIC SYSTEM

A consensus exists on the anatomical organization of the central cholinergic system. It stems from investigations carried out about 25 years ago with the use of monoclonal antibodies to choline acetyltransferase (ChAT) first developed by Kimura et al (1981). From his study and the works of Fibiger (1982), Mesulam et al (1983), Levey et al (1984), Mufson et al (1988) and others, the following schematic description can be presented:

1) Forebrain cholinergic neurons, forming a series of nuclei in the medial septum, the diagonal band of Broca and the basal magnocellular nucleus of Meynert. Since there is no precise correspondence with anatomical structures, these nuclei are frequently identified, following the classification proposed by Mesulam et al (1983), in Ch1, medial septum, Ch 2 ascending limb of the Broca's band, Ch 3 horizontal part of the band of Broca and Ch 4 nucleus of Meynert. The cholinergic neurons represent from 50 to 75% of the cells present in these nuclei and their projections form the main cholinergic afference to the cerebral cortex, hippocampus, olfactory bulb and amygdala.

2) The cholinergic interneurons of the caudate nucleus and putamen

3) The cholinergic nuclei of the brain stem including Ch 5 in the tegmental pontine nucleus, Ch 6 in the dorsolateral tegmental nucleus, Ch 7 in the medial habenular nucleus and Ch 8 in the parabigeminal nucleus. Ch5 and Ch6 project to the thalamus, hypothalamus, pallidus and to the forebrain cholinergic nuclei, Ch 7 projects to the interpeduncular nucleus and Ch 8 to the superior colliculus.

4) The motor neurons of the spinal cord.

DISEASES WITH DEGENERATION OF CHOLINERGIC NEURONS.

Alzheimer's Disease

AD is the first neurodegenerative disease in which an extensive degeneration of the cholinergic neurons was observed. Davies and Maloney (1976) reported that in post-mortem brains from AD patients there was a marked reduction in ChAT which is responsible for acetylcholine (ACh) synthesis from its immediate precursors, choline and acetyl-coA. The finding was repeatedly confirmed (Perry et al, 1977, Pepeu et al, 1979, for reviews see Bartus et al, 1982, Hardy et al, 1985). A few years later, a 75% loss of cholinergic neurons in the forebrain cholinergic neurons of AD patients was detected (Whitehouse et al, 1982, Nagai et al, 1983) demonstrating that the decrease in ChAT activity in the cerebral cortex and hippocampus in AD depends on the degeneration of cholinergic nerve endings originating from cells located in the basal forebrain and septum. The degeneration of the cortical cholinergic nerve endings is confirmed by the decrease in the vesicular ACh transporter (Efange et al, 1997) and the loss of M2 muscarinic receptors in post-mortem samples taken from AD patients (Mash et al, 1985). In the brain, M2 receptors are mostly located presynaptically and regulate ACh release (Zhang et al, 2002). The cortical cholinergic denervation in AD was confirmed by

in vivo mapping using computed tomography and [^{123}I] iodobenzovesamicol as in vivo marker of the vesicular ACh transporter (Kuhl et al, 1996). Moreover, a highly significant linear relationship was found in autopsy samples between cortical gray matter volume and nucleus basalis (NB) cell number in controls and AD patients (Cullen et al, 1997).

The amyloid cascade hypothesis (Hardy and Allsop 1991; Hardy and Higgins, 1992) considers the deposition of the peptide β-amyloid (Aβ) the main pathogenetic event of AD. Much evidence demonstrate the toxicity of Aβ for the cholinergic neurons, as reported in the review by Pakaski and Kalman (2008). In our laboratory, we demonstrated that preaggregated Aβ injections in the NB of adult rats is followed by a decrease in the number of cholinergic neurons, identified by ChAT immunostaining, and an extensive glial reaction (Giovannelli et al, 1995). The number of ChAT-immunopositive neurons is significantly reduced in the forebrain of transgenic mice exhibiting extensive cerebral Aβ deposition and glial activation (Bellucci et al, 2006).

Two questions arise: 1) are all brain cholinergic neurons equally affected in AD ? 2) through which mechanism is Aβ causing the degeneration of the cholinergic neurons which appear to be more vulnerable than other types of neurons in AD ?

In contrast with the well demonstrated degeneration of the forebrain cholinergic neurons, Woolf et al (1989) found in post-mortem brains of AD patients that the neurons of the pontomesencephalo-tegmental (PMT) cholinergic nuclei, C5 – C8, (Mesulam et al, 1983, Mufson et al, 1988) do not degenerate in AD. In the striatum of AD patients a loss of ChAT activity and a decrease in the number of ChAT-positive neurons in the caudate nucleus has been reported (Perry et al, 1977, Rossor et al, 1982a), whereas in the putamen the cholinergic neurons are not affected (Rossor et al, 1982b; Nagai et al, 1983). Some decrease in ChAT activity in the anterior and posterior grey matter of the lumbar spinal cord has been described (Yates et al, 1989) and a dysfunction of the spinal motor neurons has been reported (Sica et al, 1998). However, there are no reports describing degeneration and loss of the ChAT-positive spinal motor neurons in AD.

The reasons of these remarkable regional differences in the degeneration and loss of cholinergic neurons in AD are not yet fully understood. Woolf et al (1989) observed that the number of plaques and tangles, the histopathological landmarks of AD, is smaller in the brain stem than in the cerebral cortex, and amygdala of subjects affected by AD and therefore the brain stem neurons are less affected by the disease. More important is the observation that the basal forebrain cholinergic neurons bind NGF whereas PMT cholinergic neurons do not, although phenotypically similar (Richardson et al, 1986). According to Kordover et al (1988) there is an extensive NGF binding to ChAT-positive cells of the Ch1 – Ch4 regions whereas the binding density in the putamen is much lower. Woolf et al, (1989) found that approximately 92% of all cholinergic neurons in the basal forebrain possess receptors for NGF but these receptors were not found in association with ChAT-positive somata in the pedunculopontine and laterodorsal tegmental nuclei. Pioro and Cuello (1990) reported that the degree of overlap between NGF receptor- and ChAT -containing regions in the brainstem is not as great as in the forebrain.

NGF binds to two completely different cell surface receptors—the Trk tyrosine kinase receptors, namely TrkA, and the shared p75(NTR) receptor (Roux and Barker, 2002) with high and low affinity, respectively. The expression of TrkA mRNA was found to be restricted to neurons of the basal forebrain, caudate-putamen with features of cholinergic cells and to magnocellular neurons of several brainstem nuclei (Merlio et al, 1992). The differences in NGF receptor expression of the cholinergic neurons in different brain regions offer a basis for their different dependence on NGF supply during adulthood for the maintenance of their biochemical and morphological phenotype. Basal forebrain cholinergic neurons are greatly reduced in adult mice in which phenotypic knockout of NGF was achieved by expressing transgenic anti-NGF antibodies (Ruberti et al, 2000). Unfortunately, the brain stem cholinergic neurons were not examined but, since the mice motility was not impaired, we may assume that the motor neurons are much less NGF dependent. The trophic importance of NGF for the cholinergic forebrain neurons was also confirmed by the observation that intracerebroventricular administration of NGF ameliorates their age-

associated atrophy in old rats (Fisher et al, 1987). Moreover, NGF infusions prevent the degeneration of the ChAT-positive neurons in the NB induced by local excitotoxin injection in aging rats (Casamenti et al, 1994).

Since the constant presence of NGF is necessary for the survival of the forebrain cholinergic neurons, the question arises whether their degeneration in AD depends on a reduced availability of NGF. According to Mufson et al (2003), brain NGF levels remain stable throughout the course of AD and appear to be sufficient to support the cholinergic plasticity changes occurring during the initial phases of the disease. On the basis of post-mortem studies and animal experiments, Cuello et al (2010) propose that in AD a NGF deficit results from a dysregulation of the NGF maturation cascade caused by an impaired conversion of proNGF to mature NGF and an acceleration of mature NGF degradation. According to Bruno et al (2009), the dysregulation detected in postmortem brains of AD patients can be reproduced in naïve rats by the intracerebral injection of Aβ oligomers causing microglial activation and the ensuing release of inflammatory factors. Therefore, according to the pathogenetic mechanism proposed by Cuello et al (2010), the degeneration of the forebrain cholinergic neurons in AD begins with the canonical excess in Aβ formation and deposition associated with an extensive neuroinflammatory response which leads to NGF metabolism dysregulation. This mechanism may also explain the cholinergic neuron degeneration induced in adult rats by NB injection of preaggregated Aβ . The degeneration is associated with microglia, astrocyte activation and a strong inflammatory reaction characterized by IL-1ß production and an increased inducible cyclooxygenase and nitric oxide synthase expression (Giovannini et al, 2002).

ProNGF, whose increase was observed in postmortem AD brains (Bruno et al, 2009) and in the hippocampus of subjects with mild cognitive impairment (MCI) and AD (Mufson et al, 2012), has been shown to mediate cell death in PC12 cells (Armugan et al, 2012) and oligodendrocytes (Beattie et al, 2002), through an apoptotic mechanism involving P75(NTR) receptors. Moreover, NB cholinergic neurons in subjects affected by mild to moderate AD, displayed a significant down regulation of TrkA, TrkB and TrkC expression during the progression of the disease whereas no change in p75(NTR) expression was detected (Ginsberg et al, 2006). Trk downregulation was associated with the cognitive decline. Mufson et al (2007), comparing post mortem samples of subjects with no cognitive impairment, MCI and early AD, observed that, although the number of ChAT-positive neurons in the NB was not significantly different, there was a significant reduction in the number of TrkA but not p75(NTR) receptor-containing neurons, which co-localize with ChAT, in the MCI and early AD brains. This finding indicates that in the initial AD stages there is a decrease in the response of the cholinergic neurons to neurotrophic factors. They also observed an increase in proNGF in the cortex of subjects with MCI and early AD. Since proNGF accumulates in the presence of reduced cortical TrkA and high level of p75(NTR) receptors, a shift between molecules facilitating survival and damaging molecules seems to take place in prodromal AD. The degeneration of the forebrain cholinergic neurons may therefore be caused by a decrease in NGF availability and number of Trk binding sites, resulting in a loss of trophic effect, compounded with a proNGF apoptotic effect mediated through the binding to p75(NTR) receptors.

Using rat cortical neurons and NIH-3T3 cell line engineered to stably express p75(NTR), Yaar et al (1997) demonstrated that the Aβ peptide specifically binds to p75(NTR). Furthermore, 3T3 cells expressing p75NTR , but not wild-type control cells lacking the receptor, undergo apoptosis in the presence of aggregated Aβ. By using neuroblastoma cell clones engineered to express p75(NTR), Perini et al (2002) showed that p75(NTR) is involved in the direct signaling of cell death caused by Aβ via the function of its death domain. This signaling leads to the activation of caspases-8 and -3, the production of reactive oxygen intermediates and the induction of an oxidative stress. They also showed that the direct mechanism of neuronal damage activated by Aβ acts synergistically with the inflammatory reaction induced by Aβ. Indeed, TNF-α and IL-1β, cytokines produced by Aβ -activated microglia, may potentiate the neurotoxic action of Aβ mediated by p75(NTR) signaling. These results indicate that neurons expressing p75(NTR), if expressing

also proinflammatory cytokine receptors, may be preferential targets of the cytotoxic action of Aβ in AD. The authors suggest that the high level of expression of p75(NTR) of the basal forebrain cholinergic neurons may be the reason of their vulnerability in AD, whereas the cholinergic neurons of the brainstem, which do not express p75(NTR), remain undamaged.

The deletion of p75(NTR) receptor in a transgenic model of AD (Thy1-hAPP(Lond/Swe) x p75(NTR-/-) mice) significantly diminished hippocampal neuritic dystrophy and completely reversed the basal forebrain cholinergic neurite degeneration in comparison with AD mice expressing wild-type p75(NTR). Aβ levels were not affected, suggesting that removal of p75(NTR) extracellular domain reduced the ability of excess Aβ to promote neuritic degeneration (Knowles et al, 2009). These findings indicate that although p75(NTR) likely does not mediate all Aβ effects, it does play a significant role in enabling Aβ-induced neurodegeneration in vitro and in vivo. Further references on the interaction between Aβ and p75(NTR) receptors can be found in a recent review by Patel and Jhamandas (2012).

Concluding this paragraph, we may assume, on the basis of the results reported above, that neurodegeneration of the cholinergic neurons in AD is caused by an Aβ overload which exerts a direct toxic effect through p75(NTR) and an indirect effect through the inflammatory reaction. The products of neuroinflammation reduce the availability of NGF needed for the cholinergic neurons survival, increase the level of proNGF which in turn exerts a toxic effect on the cholinergic neurons, and synergize the toxicity of Aβ. The difference in the expression of p75(NTR) among cholinergic neurons may explain the higher vulnerability of the forebrain cholinergic neurons in comparison to the spinal and brain stem cholinergic neurons. The pivotal role of the inflammatory reaction in the degeneration of the cholinergic neurons induced by Aβ is confirmed by the finding that chronic lipopolysaccharide infusions produced a time-dependent, but not dose-dependent, decrease in cortical ChAT activity that paralleled a decline in the number of ChAT - and p75-immunoreactive cells and a dense distribution of reactive astrocytes and microglia within the basal forebrain (Willard et al, 1999). Moreover, in the rat, the anti-inflammatory drug rofecoxib, a selective cyclooxygenase-2 inhibitor suppresses brain inflammation and protects the forebrain cholinergic neurons from the degeneration induced by Aβ injection into the NB (Giovannini et al, 2003). Finally, using tissue from subjects with no cognitive impairment, MCI, and AD and a double staining for visualizing phosphorylated tau protein and p75(NTR) expressing cells, it has been shown (Vana et al, 2011) that the increase in the number of neurons of the NB showing accumulation of tau protein is accompanied by a decrease in that of cholinergic neurons identified by p75(NTR) immunostaining. This finding suggests that also the neurofibrillary tangles within the cholinergic neurons may play a role in their degeneration during AD.

It is pertinent to remind that the degeneration of the cholinergic neurons in AD, although an important and characteristic feature of this disease, is accompanied by a diffuse neuronal damage and synaptic loss (Hardy et al, 1985; Hamos et al, 1989) involving other neurotransmitter systems (Zweig et al, 1988; Halliday et al, 1992; Lai et al, 2007), presumably caused by the Aβ peptide toxicity acting directly and indirectly through the products of the associated inflammatory reaction.

Other dementias

Although PD is considered a motor disease characterized by a degeneration of dopaminergic neurons, it may present also non motor symptoms including cognitive deficits and dementia. A moderate Aβ load was demonstrated in vivo by [^{11}C] PIB positron emission tomography (PET) in the cortex and subcortical structure in PD brains (Edison et al, 2008), in association with Lewy's bodies (see below) which are a characteristic feature of PD (Spillantini, 1999).

A loss of cells in the NB was described by Whitehouse et al (1983) in postmortem brains of 12 subjects affected by PD and this finding was repeatedly confirmed (see ref in Bigl et al, 1990, Tiraboschi et al, 2000). In vivo demonstration of the cholinergic denervation was obtained with neuroimaging techniques using ligands for vesicular ACh transport (VAChT) and acetylcholinesterase (AChE) (see ref. in Bohnen and Albin, 2011) A denervation of the limbic archicortex in PD patients was demonstrated by PET using [^{11}C]methyl-4-piperidinyl propionate

to visualize AChE and the level of denervation correlated with olfactory dysfunction (Bohnen et al, 2010). A thalamic cholinergic denervation was demonstrated (Kotagal et al, 2012) in subjects affected by PD, PD with dementia (PDD), and Lewy body dementia (LBD) but not in AD. Hilker et al (2005) reported significant reductions of cortical AChE in PD without dementia but severe reductions in PD with dementia. Dementia in PD subjects is sporadic and may begin one or more years after the onset of the motor symptoms. The development of severe cognitive deficits before or together with the motor symptoms is a characteristic feature of LBD. LBD is a type of dementia closely associated with both AD and PD. It is characterized anatomically by the presence of Lewy bodies (LB) which are clumps of α synuclein and ubiquitin proteins in neurons, detectable by post mortem brain histology (Kalra et al, 1996; Perry et al, 1997, Spillantini, 1999). The Aβ load is significantly raised in most LBD cases, as demonstrated in vivo by PET visualization (Edison et al, 2008). Two forms are described, the AD variant with LB and the diffuse Lewy Body Disease and in both forms a marked decrease, up to 75%, in ChAT activity in the midfrontal cerebral cortex and in the hippocampus was observed (Tiraboschi et al, 2000). The loss of ChAT activity is less severe and occurs later in the clinical course of AD than in LBD in which ChAT loss is already prominent in the earliest stages of the illness (Tiraboschi et al, 2002).

In post mortem brains of LBD subjects, as well as in subjects with multiple system atrophy, a significant loss of cholinergic neurons was detected by Schmeichel et al (2008) in the pedunculopontine nucleus (PPN) and the laterodorsal tegmental nuclei (Ch 5, Ch 6) which are spared in AD. The degeneration of the Ch5 and Ch6 cholinergic nuclei leads to the cholinergic denervation of the thalamus observed in LBD and PD but not in AD by Kotagal et al (2012) by measuring PPN-Thalamic AChE activity by PET imaging.

A cholinergic deficit has been also observed in atypical parkinsonian diseases. Tagliavini et al (1984) and a few other authors (see ref in Bigl et al, 1990) reported a significant loss of neurons in the NB in subjects affected by progressive supranuclear palsy (PSP).This disease, characterized by clinical features including extrapyramidal symptoms, ocular dyscontrol and cognitive impairment, is considered a tauopathy for the extensive neurofibrillary cluster of phosphorylated tau protein detected in the brains (Boewe, 2012). VAChT expression and ChAT activity in caudate nucleus and putamen were also found to be markedly decreased in postmortem brains of subject with PSP, consistent with a selective loss of striatal cholinergic interneurons (Suzuki et al, 2002).

Shinotoh et al (1999) reported a modest reduction in cortical AChE activity in patients with PSP, smaller than in PD subjects, and a 38% reduction in the thalamus. The latter result indicates a significant loss of brainstem cholinergic PPN neurons. Therefore, it appears that in this atypical parkinsonian disease there is a widespread alteration of the cholinergic neurons involving the cholinergic forebrain nuclei, the striatal cholinergic interneurons and brain stem cholinergic nuclei.

The corticobasal syndrome also belongs to the atypical parkinsonian diseases and shows a decrease in AChE activity, demonstrated by neuroimaging. The decrease was observed in the paracentral region and the frontal, parietal and occipital cortices (Shinotoh et al, 1999; Hirano et al, 2010) which are projection areas of the NB cholinergic neurons. At variance with PSP, with which the corticobasal syndrome is frequently confused (Stripp, 2011), no reduction in thalamic AChE activity was detected. Both diseases are defined tauopathies and are characterized by neurofibrillary pathology.

The frontotemporal dementias, which represent in prevalence the second group of senile dementias (Snowden et al, 2002) are also characterized by neurofibrillary pathology. However, no decrease in cortical or thalamic AChE was observed by neuroimaging (Hirano et al, 2010). They are characterized by personality, behavior disturbances, limited memory loss, and present several variants on the basis of the nature of the characteristic protein inclusions (Goedert et al, 2012). Pick's disease is included in the frontotemporal dementias (Kerstez, 2004). The studies of the cholinergic neurons of the NB report contrasting findings (Bigl et al 1990) ranging from no loss of cholinergic neurons to a 70% decrease. However, according to Hansen et al (1988), ChAT levels were normal in 5 cases of Pick's disease whereas they were reduced in AD cases studied by comparison.

Mechanisms responsible of the degeneration of the cholinergic neurons in Parkinson's disease and atypical Parkinsonian diseases.

As described above, in AD the loss of cholinergic neurons is confined to the cholinergic forebrain nuclei. Their degeneration is attributed to a dysregulation of NGF formation and metabolism presumably caused by Aβ toxicity and the associated extensive inflammatory response (Cuello et al, 2010; Mufson et al, 2007) and to direct Aβ toxicity. The degeneration of the cholinergic neurons in PD and the other neurodegenerative diseases which share the presence of tau neurofibrils, neurofibrillary tangles, and Lewy bodies, shows different patterns. In PD, PD with dementia and LBD there is a loss of forebrain cholinergic neurons (Bigl et al, 1990; Hiker et al, 2005; Tiraboschi et al, 2000) which may be caused by the presence of an Aβ load and Aβ plaques, particularly in LBD, through the mechanisms discussed above. However, in PD and LBD there is a degeneration of the midbrain cholinergic neurons located in Ch 5 and Ch 6 (Schmeikel et al, 2008) and a thalamic cholinergic denervation which do not occur in AD. The midbrain cholinergic neurons do not express NGF receptors (Woolf et al, 1989) and are less dependent on NGF supply. In PSP, besides the loss of the forebrain and midbrain cholinergic neurons, a degeneration of the striatal cholinergic interneurons was described (Suzuki et al, 2002). Therefore, different mechanisms should be responsible of the degeneration of the cholinergic neurons. Finally, the frontotemporal dementias, including Pick's disease, do not show a consistent loss of forebrain cholinergic neurons and a significant cortical and thalamic cholinergic denervation. Since the frontotemporal dementias are tauopathies characterized by neurofibrillary deposits, it appears that tau is not particularly toxic for the cholinergic neurons, even if its neurotoxicity is well documented in hippocampal organotypic slice cultures (Messing et al, 2012). Indeed, no obvious differences in the distribution and density of cholinergic and monoaminergic neurons were found comparing tau filament forming transgenic mice with wild type mice (Morcinek et al, 2012). Conversely, in A30P α-synuclein-expressing transgenic mice, a degeneration of the forebrain cholinergic neurons was observed after dopamine depletion induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration (Szego et al, 2011). This observation was confirmed and extended by Szego et al (2013) who reported that the number of neurons expressing a cholinergic marker in the medial septum-diagonal band of Broca complex decreases in A30P α-synuclein-expressing mice during aging, paralleled by a lower AChE fiber density in the dentate gyrus and in the hippocampal CA1 field. After inducing dopamine depletion by MPTP, no acute but a delayed loss of cholinergic neurons and AChE-positive fibers was observed, which was attenuated by L-3,4-dihydroxyphenylalanine (DOPA) treatment. However, P301L tau transgenic pR5 mice, overexpressing α-synuclein, develop neurofibrillary lesions but do not show the degeneration of basal forebrain cholinergic neurons observed in Alzheimer's disease (Koehler et al, 2010). It appears that α-synuclein toxicity for the cholinergic neurons is reinforced by age and dopamine depletion, a situation occurring in PD and parkinsonian diseases and therefore α synuclein may be responsible for the degeneration of the midbrain cholinergic neurons and contribute to that of the forebrain neurons.

Alcoholic dementia

Chronic ethanol abuse may lead to alcoholic dementia and the related Korsakoff's syndrome and Wernicke's encephalopathy (WE), whose cognitive deficits mimic AD. The first observation of a loss of cholinergic neurons in the NB of subjects affected by Korsakoff's syndrome was made by Arendt et al (1983). No significant decrease in the number of cholinergic neurons was found in chronic alcoholism without dementia. The loss of cholinergic neurons concurs with the large decrease in ChAT activity detected in the cortex, hippocampus and cerebellum of subjects with alcoholic dementia by Antuono et al (1980). The degeneration of the cholinergic neurons caused by ethanol abuse can be reproduced in the rat. After 6 months of ethanol intake, a loss of cholinergic neurons, affecting the forebrain nuclei, but sparing the brain stem Ch 5 and Ch6 nuclei, was observed by Arendt et al (1988) and was accompanied by a decrease in AChE activity in the cerebral cortex, hippocampus and amygdala indicating a cholinergic denervation. In rats drinking ethanol for 6 months, the decrease in ChAT activity and ACh release in the

cortex and hippocampus, together with the cognitive impairment, was observed even after four weeks withdrawal (Casamenti et al 1993). Investigating the mechanism of the neuronal degeneration, induced by ethanol abuse, Cullen and Halliday (1995a) observed that in chronic alcoholics with thiamine deficiency, neurofibrillary pathology was found in the NB, but in no other brain regions. Neurofibrillary tangles were not seen in age-matched controls and were infrequent in alcoholics without neuropathological signs of thiamine-deficiency. Neurofibrillary tangles were most numerous in the cases showing NB cell loss. The authors concluded that NB neurodegeneration in chronic alcoholics proceeds through the formation of neurofibrillary tangles. Extending their investigations (Cullen and Halliday, 1995b), they observed that tau-positive granular and fibrillary inclusions were frequently observed within the magnocellular neurons of the NB in WE subjects, occasionally in non-WE alcoholics, and never in controls. Tau immunoreactivity was not observed in cortical, brainstem, diencephalic or non-cholinergic forebrain structures. The majority of neurons in the basal forebrain showed increased peroxidase activity in all WE alcoholics and in some NB neurons of non-WE alcoholics, but was rarely seen in controls. These results suggest a link between peroxidase activity and the abnormal accumulation of phosphorylated tau. The presence of tau in the NB of alcoholics with WE suggests a thiamine-dependent mechanism in tau accumulation and cell death in the cholinergic basal forebrain. Thiamine deficiency is a common consequence of alcohol abuse and the consequences of thiamine deficiency on ACh synthesis have been demonstrated long time ago (Heinrich et al, 1973). Thiamine deficiency is therefore an important factor in the dysfunction of the cholinergic neurons in alcoholic dementia. However, it does not explain why the degeneration occurs almost exclusively in the Ch 1–Ch 4 nuclei. A neurotoxic mechanism involving neuroinflammation and possibly NGF dysfunction may be hypothesized. It must be mentioned that in organotypic brain slices of the NB exposed to ethanol, NGF, inhibition of MAPK p38 and NOS protected the cholinergic neurons against the ethanol-induced effect (Ehrlich et al, 2012) confirming the complexity of the mechanism leading to cholinergic cell degeneration in this pathology.

CONSEQUENCES OF THE DEGENERATION OF THE BRAIN CHOLINERGIC SYSTEM.

The most evident consequence of the degeneration of the forebrain cholinergic system is a cognitive deficit. More than thirty years ago in a seminal paper Drachman (1977) asked whether the cholinergic system has a specific role in memory and cognitive functions in man. Shortly later, the cholinergic hypothesis of geriatric memory dysfunction (Bartus et al, 1982) was proposed and was followed by countless papers investigating and demonstrating the role of the cholinergic system in learning and memory. An analysis of these papers is beyond the scope of this article and therefore we refer the reader to a few recent reviews (Hasselmo, 2006 Pepeu and Giovannini, 2006, Woolf and Butcher, 2010; Benarroch, 2010; Hasselmo and Sarter, 2011). ACh in the brain alters neuronal excitability, influences synaptic transmission, induces synaptic plasticity, and coordinates firing of groups of neurons. As a result, it changes the state of neuronal networks throughout the brain and modifies their response to internal and external inputs (Picciotto et al, 2012). The administration of anticholinergic drugs to humans and animals, the use of cholinergic receptor knockout mice and the lesioning of the forebrain cholinergic neurons in animals result in deficit of attention, impairment in information acquisition and amnesia. Since dementias are characterized by multiple cognitive deficits including the impairment of memory (DSM IV), it may be assumed that the loss of forebrain cholinergic neurons is largely responsible for the cognitive deficits of dementias. On this assumption, cholinesterase inhibitors are used as therapeutic agents in AD with the aim to enhance the residual cholinergic function (Giacobini, 2000).

In PD and LBD a degeneration of the midbrain cholinergic neurons located in the Ch 5 and Ch 6 nuclei was observed, with or without the concomitant loss of Ch1–Ch4 neurons. The midbrain cholinergic neurons innervate the spinal cord, brain stem, thalamus, hypothalamus, basal forebrain and medial frontal cortex and are involved in arousal and attention, the sleep-wakefulness cycle and

the regulation of muscular tone during REM sleep (Woolf and Butcher, 2011). Their loss may be a cause of the sleep behavior disorders in DLB (Schmeichel et al, 2008).

Since the first observation in transfected cultured cells (Nitsch et al,1992) that stimulation of M1 and M3 muscarinic receptor subtypes increased the basal secretion of amyloid precursor protein (APP), the possible role of the brain cholinergic system in the regulation of Aβ peptide metabolism has been the object of much investigations (see ref in Pakalski and Kalman, 2008). Experiments "in vitro" and in murine AD models showed that M1 receptors have a role in APP secretion via alpha-secretase activation and in decreasing Aβ levels, via beta-secretase inhibition (Fisher et al, 2003). Therefore it has been assumed that in AD the degeneration of the cholinergic neurons, with the ensuing cholinergic hypofunction, may aggravate the Aβ overload which is considered its main pathogenetic mechanism.

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REFERENCES

Antuono P, Sorbi S, Bracco L, Fusco T, Amaducci L (1980) A discrete sampling technique in senile dementia of the Alzheimer type and alcoholic dementia: study of the cholinergic system. In: Amaducci L, Davison AN, Antuono P Eds Aging of the brain and dementia. Raven Press, New York 151 – 158.

Arendt T, Bigl V, Arendt A, Tennstedt A (1983) Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's Disease. *Acta Neuropathol.* **61**:101-108.

Arendt T, Henning D, Gray JA, Marchbanks R (1988) Loss of neurons in the rat basal forebrain cholinergic projection system after prolonged intake of ethanol. *Brain Res Bull.* **21**:563-569.

Armugam A, Koh DC, Ching CS, Chandrasekaran K,et al and Jeyaseelan K (2012) Pro-domain in precursor nerve growth factor mediates cell death. *Neurochem Int.* **60**:852-563.

Ballmaier M, Casamenti F, Zoli M, Pepeu G, Spano P (2001) Selective immunolesioning of cholinergic neurons in nucleus basalis magnocellularis impairs prepulse inhibition of acoustic startle. *Neuroscience* **108**:299-305.

Bartus RT, Dean RL 3rd, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**:408-414.

Beattie MS, Harrington AW, Lee R, Kim JY et al and Yoon SO (2002) ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron* **36**:375–386.

Bellucci A, Luccarini I, Scali C, Prosperi C, Giovannini MG, Pepeu G, Casamenti F (2006) Cholinergic dysfunction, neuronal damage and axonal loss in TgCRND8 mice. *Neurobiol Dis* **23**: 260 – 272.

Benarroch EE (2010) Acetylcholine in the cerebral cortex: effects and clinical implications. *Neurology* **75**: 659 – 665.

Bigl V, Arendt T, Biesold D (1990) The nucleus basalis of Meynert during ageing and in dementing neuropsychiatric disorders. In: Steriade M, Biesold D, Eds Brain cholinergic systems. Oxford University Press, Oxford: 364 – 386.

Bohnen NI, Müller ML, Kotagal V, Koeppe RA, Kilbourn MA, Albin RL, Frey KA (2010) Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease. *Brain* **133**:1747-1754.

Bohnen NI, Albin RL (2011) The cholinergic system and Parkinson disease. *Behav Brain Res* **221**:564-73.

Boeve BF (2012) Progressive supranuclear palsy. Parkinsonism Relat Disord 18 Suppl 1:S192-194.

Bruno MA, Leon WC, Fragoso G, Mushynski WE, Almazan G, Cuello AC (2009) Amyloid beta-induced nerve growth factor dysmetabolism in Alzheimer disease. *J Neuropathol Exp Neurol* **68**:857-869.

Casamenti F, Scali C, Vannucchi MG, Bartolini L, Pepeu G (1993) Long term ethanol consumption by rats: effect on acetylcholine release in vivo, choline acetyltransferase activity, and behavior. *Neuroscience* **56**: 465 – 471.

Casamenti F, Scali C, Giovannelli L, Faussone-Pellegrini MS, Pepeu G (1994) Effect of nerve growth factor and GM1 ganglioside on the recovery of cholinergic neurons after a lesion of the nucleus basalis in aging rats. *J Neural Transm* [P-D Sect] **7**: 177 -193.

Cuello AC, Bruno MA, Allard S, Leon W, Iulita MF (2010) Cholinergic involvement in Alzheimer's disease. A link with NGF maturation and degradation. J Mol Neurosci 40: 230 – 235.

Cullen KM, Halliday GM (1995a) Neurofibrillary

tangles in chronic alcoholics. *Neuropathol Appl Neurobiol* **21**:312-318.

Cullen KM, Halliday GM (1995b) Mechanisms of cell death in cholinergic basal forebrain neurons in chronic alcoholics. *Metab Brain Dis* **10**:81-91.

Cullen KM, Halliday GM, Double KL, Brooks WS, Creasey H, Broe GA (1997) Cell loss in the nucleus basalis is related to regional cortical atrophy in Alzheimer's disease. *Neuroscience* **78**: 641 - 652.

Drachman DA (1977) Memory and cognitive function in man: does the cholinergic system have a specific role ? *Neurology* **27**: 783 -790.

DSM IV (1994) American Psychiatric Association, Washington DC, 123

Edison P, Rowe CC, Rinne JO, Ng S, et al, and Brooks DJ. (2008) Amyloid load in Parkinson's disease dementia and Lewy body dementia measured with [^{11}C] PIB positron emission tomography. *J Neurol Neurosurg Psychiatry* **79**:1331–1338.

Efange SMN, Garland EM, Staley JK, Khare AB, Mash DC (1997) Vesicular acetylcholine transporter density and Alzheimer's disease. *Neurobiol Aging* **18**: 407 – 413.

Hasselmo ME (2006) The role of acetylcholine in learning and memory. *Curr Opin Neurobiol* **16**:710-715.

Hasselmo ME, Sarter M (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* **36**:52-73.

Heinrich CP, Stadler H, Weiser H (1973) The effect of thiamine deficiency on the acetilcoenzyme A and acetylcholine levels in the rat brain. *J Neurochem* **21**: 1273 – 1281.

Ehrlich D, Pirchl M, Humpel C (2012) Ethanol transiently suppresses choline-acetyltransferase in basal nucleus of Meynert slices. *Brain Res* **1459**:35-42.

Fibiger HC (1982) The organization and some projections of cholinergic neurons of the mammalian forebrain. *Brain Res* **257**:327-388.

Fisher W, Wictorin K, Bjorklund A, Williams LR, Varon S, Gage FH (1987) Amelioration of cholinergic neurons atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature* **329**: 65-68.

Fisher A, Pittel Z, Haring R, Bar-Ner N, et al and Brandeis R. (2003) M1 muscarinic agonists can modulate some of the hallmarks in Alzheimer's disease: implications in future therapy. *J Mol Neurosci* **20**:349-356.

Giacobini E. (2000) Cholinesterase inhibitors: from the Calabar bean to Alzheimer's disease. In: Giacobini E, Ed, 'Cholinesterases and cholinesterase inhibitors'. Martin Dunitz, London, 181-226.

Ginsberg SD, Che S, Wuu J, Counts SE, Mufson EJ (2006) Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. *J Neurochem* **97**:475-487.

Giovannelli L, Casamenti F, Scali C, Bartolini L, Pepeu G (1995) Differential effects of amyloid peptides β-(1– 40) and β-(25–35) injections into the rat nucleus basalis. *Neuroscience* **66**: 781 -792.

Giovannini MG, Scali C, Prosperi C, Bellucci A et al and Casamenti F (2002) Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: involvement of the p38MAPK pathway. *Neurobiol Dis* **11**:257-274.

Giovannini MG, Scali C, Prosperi C, Bellucci A, Pepeu G, Casamenti F (2003) Experimental brain inflammation and neurodegeneration as model of Alzheimer's disease: protective effects of selective COX-2 inhibitors. *Int J Immunopathol Pharmacol* **16**: 31 – 40.

Goedert M, Ghetti B, Spillantini MG (2012) Frontotemporal dementia: implications for understanding Alzheimer disease. Cold Spring Harb Perspect Med 2:a006254. doi: 10.1101/cshperspect.a006254.

Halliday GM, McCann HL, Pamphlett R, Brooks WS et al and Harper CG (1992) Brain stem serotonin-synthesizing neurons in Alzheimer's disease: a clinicopathological correlation. *Acta Neuropathol* **84**:638-650.

Hamos JE, De Gennaro LJ, Drachman DA (1989) Synaptic loss in Alzheimer's disease and other dementias. *Neurology* **39**: 355 – 361.

Hardy J, Adolfsson R, Alafuzoff I, Bucht G et al and Winblad B (1985) Transmitter deficits in Alzheimer's disease. *Neurochem Int* **7**:545-563.

Hardy J, Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci* **12**:383 -388.

Hardy J, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**: 184 – 185.

Hilker R, Thomas AV, Klein JC, Weisenbach S et al and Heiss WD.(2005) Dementia in Parkinson disease: functional imaging of cholinergic and dopaminergic pathways. *Neurology*. **65**:1716–1722.

Hirano S, Shinotoh H, Shimada H, Aotsuka A, et al and Suhara T (2010) Cholinergic imaging in corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia. *Brain* **133**: 2058-2068.

Hornykiewicz O (1971) Pharmacology and pathophysiology of dopaminergic neurons. *Adv Cytopharmacol* **1**:369-377.

Kalra S, Bergeron C, Lang AE (1996) Lewy body disease and dementia. A review. *Arch Intern Med.* **156**:487-493.

Kimura H, McGeer PL, Peng JH, McGeer EG. (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. *J Comp Neurol.* **200**:151-201.

Knowles JK, Rajadas J, Nguyen TV, Yang T, et al and Longo FM (2009) The p75 neurotrophin receptor promotes amyloid-beta(1-42)-induced neuritic dystrophy in vitro and in vivo. *J Neurosci.* **29**:10627-10637.

Kordower JH, Bartus RT, Bothwell M, Schatteman G, Gash DM (1988) Nerve growth factor receptor immunoreactivity in the nonhuman primate (Cebus apella):distribution, morphology, and colocalization with cholinergic enzymes. *J Comp Neurol.* **277**:465-486.

Kotagal V, Müller ML, Kaufer DI, Koeppe RA, Bohnen NI (2012)Thalamic cholinergic innervation is spared in Alzheimer disease compared to parkinsonian disorders. *Neurosci Lett* **512**: 169-172.

Kuhl DE, Minoshima S, Fessler JA, Frey KA, et al and Koeppe RA (1996) In vivo mapping of cholinergic terminals in normal aging, Alzheimer's disease, and Parkinson's disease. *Ann Neurol.* **40**:399-410.

Lai MKP, Ramirez KJ, Tsang SWY, Francis P. (2007) Alzheimer's disease as a neurotransmitter disease. In: Dawbarn D and Allen SJ Eds Neurobiology of Alzheimer's disease. Oxford University Press, Oxford, 244 – 282.

Levey AI, Wainer BH, Rye DB, Mufson EJ, Mesulam MM (1984) Choline acetyltransferase-immunoreactive neurons intrinsic to rodent cortex and distinction from acetylcholinesterase-positive neurons. *Neuroscience* **13**:341-53.

Mantione CR, Fisher A, Hanin I (1981) The AF64a-treated mouse: possible model for central cholinergic hypofunction. *Science* **213**:579-580.

Mash DC, Flynn DD, Potter LT (1985) Loss of M2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science* **228**:1115-1117.

Merlio JP, Ernfors P, Jaber M, Persson H (1992) Molecular cloning of rat trkC and distribution of cells expressing messenger RNAs for members of the trk family in the rat central nervous system. *Neuroscience* **51**:513-532.

Mesulam MM, Mufson EJ, Levey AI, Wainer BH (1983) Cholinergic innervations of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area. Diagonal band nuclei, nucleus basalis (substantia innominata) and hypothalamus in the Rhesus monkey. *J. Comp. Neurol.* **214**:170 – 197.

Morcinek K, Köhler C, Götz J, Schröder H (2012) Pattern of tau hyperphosphorylation and neurotransmitter markers in the brainstem of senescent tau filament forming transgenic mice. Brain Res. pii: S0006-8993(12)01906-3. doi: 10.1016/j.brainres.2012.12.016 [Epub ahead of print].

Mufson EJ, Martin TL, Mash DC, Wainer BH, Mesulam MM (1986) Cholinergic projections from the parabigeminal nucleus (Ch8) to the superior colliculus in the mouse: a combined analysis of horseradish peroxidase transport and choline acetyltransferase immunohistochemistry. *Brain Res* **370**:144-148.

Mufson EJ, Brashers-Krug T, Kordower JH. (1992) p75 nerve growth factor receptor immunoreactivity in the human brainstem and spinal cord. *Brain Res* **589**:115-23.

Mufson EJ, Ikonomovic MD, Styren SD, Counts SE, et al and DeKosky ST(2003). Preservation of brain nerve growth factor in mild cognitive impairment and Alzheimer disease. *Arch Neurol* **60**:1143-1148.

Mufson EJ, Counts SE, Fahnestock M, Ginsberg SD (2007) Cholinotrophic molecular substrates of mild cognitive impairment in the elderly. *Curr Alzheimer Res* **4**:340-350.

Mufson EJ, He B, Nadeem M, Perez SE, Counts SE et al and Scheff SW(2012) Hippocampal ProNGF signaling pathways and β-amyloid levels in mild cognitive impairment and Alzheimer disease. *J Neuropathol Exp Neurol* **71**:1018-1029.

Nagai T, McGeer PL, Peng JH, McGeer EG, Dolman CE (1983) Choline acetyltransferase immunohistochemistry in brains of Alzheimer's disease patients and controls. *Neurosci Lett* **36**:195-199.

Nitsch RM, Slack BE, Wurtman RJ, Growdon JH (1992) Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* **258**:304-307.

Olton DS, Wenk GL (1987) Dementia: animal models of the cognitive impairments produced by degeneration of the basal forebrain cholinergic system. In: Psychopharmacology The third generation of progress. Meltzer HY Ed, Raven Press New York, 941 – 953.

Pakaski M, Kalman J (2008) Interaction between the amyloid and cholinergic mechanisms in Alzheimer's disease. *Neurochem Int* **53**: 103 – 111.

Patel AN, Jhamandas JH (2012) Neuronal receptors as targets for the action of amyloid beta protein (Aβ) in the brain. *Expert Reviews in Molecular Medicine* **14**: 1-18.

Pepeu G, Gori G, Antuono P (1979) Changes in brain neurotransmitters during aging and cerebral hypoxia. In: Drug treatment and prevention in cerebrovascular disorders. Tognoni G, Garattini S Eds, Elsevier/North Holland, Amsterdam 3 - 12.

Pepeu G, Giovannini MG (2006) The role of the cholinergic system in cognitive processes. In: The brain cholinergic system in health and disease. Giacobini E and Pepeu G Eds, Informa Healthcare, Abingdon, 221 – 233.

Perini G, Della-Bianca V, Politi V, Della Valle G, Dal-Pra I, Rossi F, Armato U (2002) Role of p75 neurotrophin receptor in the neurotoxicity by beta-amyloid peptides and synergistic effect of inflammatory cytokines. *J Exp Med* **195**: 907-918.

Perry EK, Perry RH, Blessed G, Tomlinson BE (1977) Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* Jan 22, **189**.

Perry R, McKeith I, Perry E (1997) Lewy body dementia-clinical, pathological and neurochemical interconnections. *J Neural Transm Suppl* **51**:95-109.

Picciotto MR, Higley MJ, Mineur YS (2012) Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron* **76**:116-129.

Pioro EP, Cuello AC (1990) Distribution of nerve growth factor receptor-like immunoreactivity in the adult rat central nervous system. Effect of colchicine and correlation with the cholinergic system II. Brainstem, cerebellum and spinal cord. *Neuroscience* **34**: 89-110.

Richardson PM, Verge Issa VMK, Riopelle RJ (1986) Distribution of neuronal receptors for nerve growth factor in the rat. *J Neurosci* **6**: 2312 -2321.

Rossor MN, Svendsen C, Hunt SP, Mountjoy CQ, Roth M, Iversen LL (1982a) The substantia innominata in Alzheimer's disease: an histochemical and biochemical study of cholinergic marker enzymes. *Neurosci Lett* **28**: 217 – 222.

Rossor MN, Garrett NJ, Johnson AL, Mountjoy CQ, Roth M, Iversen LL (1982b) A post-mortem study of the cholinergic and GABA systems in senile dementia. *Brain* **105**: 313 -330.

Roux PP, Barker PA (2002) Neurotrophin signaling through the p75 neurotrophin receptor. *Prog Neurobiol* **67**: 203-233.

Ruberti F, Capsoni S, Comparini A, Di Daniel E et al and Cattaneo A (2000) Phenotypic knockout of nerve growth factor in adult transgenic mice reveals severe deficits in basal forebrain cholinergic neurons, cell death in the spleen, and skeletal muscle dystrophy. *J Neurosci* **20**: 2589-2601.

Schmeichel AM, Buchhalter LC, Low PA, Parisi JE, Boeve BW, Sandroni P, Benarroch EE (2008) Mesopontine cholinergic neuron involvement in Lewy body dementia and multiple system atrophy. *Neurology* **70**: 368-373.

Shinotoh H, Namba H, Yamaguchi M, Fukushi K et al and Irie T (1999) Positron emission tomographic measurement of acetylcholinesterase activity reveals differential loss of ascending cholinergic systems in Parkinson's disease and progressive supranuclear palsy. *Ann Neurol* **46**:62–69.

Sica RE, Pereyra R, Mangone CA(1998) Loss of motor units in Alzheimer's disease. Electromyogr Clin Neurophysiol 38:475 -479.

Snowden JS, Neary D, Mann DM (2002) Frontotemporal dementia. Br J Psychiatry 180:140-143.

Spillantini MG (1999) Parkinson's disease, dementia with Lewy bodies and multiple system atrophy are alpha-synucleinopathies. *Parkinsonism Relat Disord* **5**:157-162.

Strupp M. (2011) Corticobasal syndrome: a field of uncertainty. *J Neurol* **258**:173-175.

Svensson AL, Giacobini E (2000) Cholinesterase inhibitors do more than inhibit cholinesterase. In: Giacobini E Ed, Cholinesterases and cholinesterase inhibitors. Martin Dunitz, London, 227-235.

Suzuki M, Desmond TJ, Albin RL, Frey KA (2002) Cholinergic vesicular transporters in progressive supranuclear palsy. *Neurology* **58**:1013–1018.

Szego ÉM, Gerhardt E, Outeiro TF, Kermer P (2011) Dopamine-depletion and increased α-synuclein load induce degeneration of cortical cholinergic fibers in mice. *J Neurol Sci.* **310**:90-95.

Szegő ÉM, Outeiro TF, Kermer P, Schulz JB (2013) Impairment of the septal cholinergic neurons in MPTP-treated A30P α-synuclein mice. *Neurobiol Aging* **34**: 589-601.

Tagliavini F, Pilleri G, Bouras C, Costantinids J (1984) The basal nucleus of Meynert in patients with progressive supranuclear palsy. *Neurosci Lett* **44**: 37–42.

Tiraboschi P, Hansen LA, Alford M, Sabbagh MN, Schoos B, Masliah E, Thal LJ, Corey-Bloom J. (2000) Cholinergic dysfunction in diseases with Lewy bodies. *Neurology* **54**:407-411.

Tiraboschi P, Hansen LA, Alford M, Merdes A, Masliah E, Thal LJ, Corey-Bloom J (2002) Early and widespread cholinergic losses differentiate dementia

with Lewy bodies from Alzheimer disease. *Arch Gen Psychiatry* **59**: 946-951.

Vana L, Kanaan NM, Ugwu IC, Wuu J, Mufson EJ, Binder LI (2011) Progression of tau pathology in cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease. *Am J Pathol* **179**: 2533-2550.

Varnum MM, Ikezu T (2012) The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer's disease brain. *Arch Immunol Ther Exp* (Warsz) **60**: 251-266.

Willard LB, Hauss-Wegrzyniak B, Wenk GL (1999) Pathological and biochemical consequences of acute and chronic neuroinflammation within the basal forebrain cholinergic system of rats. *Neuroscience* **88**: 193-200.

Wiley RG, Oeltmann TN, Lappi DA (1991) Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Res* **562**: 149-153.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**:1237-1239.

Wiss-Coray T, Mucke L (2002) Inflammation in neurodegenerative disease – a double-edged sword. *Neuron* **35**: 419 -432.

Woolf NJ, Jacobs RW, Butcher LL (1989a) The pontomesencephalotegmental cholinergic system does not degenerate in Alzheimer's disease. *Neurosci Lett* **96**: 277 – 282.

Woolf NJ, Butcher LL (2011) Cholinergic systems mediate action from movement to higher consciousness. Behav Brain Res 221:488-498.

Woolf NJ, Gould E, Butcher LL (1989b). Nerve growth factor receptor is associated with cholinergic neurons of the basal forebrain but not the pontomesencephalon. *Neuroscience* **30**:143–152.

Yaar M, Zhai S, Pilch PF, Doyle SM, Eisenhauer PB, Fine RE, Gilchrest BA (1997) Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. *J Clin Invest* **100**: 2333-2340.

Yates CM, Simpson J, Gordon A, Christie JE (1989) Cholinergic enzymes in the spinal cord in Alzheimer-type dementia. *J Neural Transm* (P - D Sect)**1**: 311-315.

Zhang W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess J (2002) Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *J Neurosci* **22**: 1709-1717.

Zweig RM, Ross CA, Hedreen JC, Steele C et al and Price DL (1988) The neuropathology of aminergic nuclei in Alzheimer's disease. *Ann Neurol* **24**:233 - 242.