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Nicotinic receptor subtypes in human brain ageing, Alzheimer and Lewy body diseases

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Abstract

Human brain ageing is associated with reductions in a variety of nicotinic receptors subtypes, whereas changes in age-related disorders including Alzheimer's disease or Parkinson's disease are more selective. In Alzheimer's disease, in the cortex there is a selective loss of the α4 (but not α3 or 7) subunit immunoreactivity and of nicotine or epibatidine binding but not α-bungarotoxin binding. Epibatidine binding is inversely correlated with clinical dementia ratings and with the level of Aβ1–42, but not related to plaque or tangle densities. In contrast, α-bungarotoxin binding is positively correlated with plaque densities in the entorhinal cortex. In human temporal cortex loss of acetylcholinesterase catalytic activity is positively correlated with decreased epibatidine binding and in a transgenic mouse model over expressing acetylcholinesterase, epibatidine binding is elevated. In Parkinson's disease, loss of striatal nicotine binding appears to occur early but is not associated with a loss of α4 subunit immunoreactivity. Tobacco use in normal elderly individuals is associated with increased α4 immunoreactivity in the cortex and lower densities of amyloid-β plaques, and with greater numbers of dopaminergic neurons in the substantia nigra pars compacta. These findings indicate an early involvement of the α4 subunit in β-amyloidosis but not in nigro-striatal dopaminergic degeneration.

Keywords: Alzheimer's disease; Parkinson's disease; α3 subunit; α4 subunit; α7 subunit; β-Amyloidosis; Acetylcholinesterase; Tobacco exposure

1. Introduction

Research on the role of neuronal nicotinic receptors in brain ageing and degenerative diseases associated with ageing such as Alzheimer's disease and dementia with Lewy bodies, Parkinson's disease, aims to determine:

1. Which nicotinic receptors subtypes are affected and how the receptor is involved in pathological changes such as system degeneration and neuron loss, β-amyloidosis, abnormal tau, Lewy body and Lewy neurite formation.

2. What is the evidence that any particular subtype contributes to the pathological cascade and that receptor

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modification will thus protect against the development of, e.g. Alzheimer's and Parkinson's diseases.

3. How receptor abnormalities are linked to specific clinical symptoms and what are the prospects for receptor-targeted symptomatic therapy.

The loss of nicotinic receptors with increasing age is one of the most consistent findings in relation to changes in the ageing human brain. Changes in the cortex or hippocampus from maturity to old age include: reductions in nicotine binding (likely to reflect $\alpha 4$ or $\alpha 3$); α-bungarotoxin binding ($\alpha 7$); and $\alpha 3$, $\alpha 4$ and $\beta 2$ mRNA (Flynn and Mash, 1986; Perry et al., 1986; Schröder et al., 1991; Court et al., 1992; Nordberg et al., 1992; Court et al., 1997; Terzano et al., 1998; Tohgi et al., 1998a,b; Utsugisawa et al., 1999). In the striatum a reduction in nicotine binding occurs only after the age of 75 years (Hellström-Lindahl and Court, in press), contrasting with the cortex and hippocampus where the receptor declines earlier. These differential patterns of age-related changes may reflect functional changes in mnemonic function (associated with the hippocampus) occurring earlier during normal ageing than in extrapyramidal motor dysfunction.

The further changes that take place in Alzheimer's and Parkinson's disease include the loss of high affinity agonist (nicotine, cytisidine or epibatidine) binding which occurs in the cortex in both diseases and also in the striatum in Parkinson's disease (Perry et al., 1995; Warpman and Nordberg, 1995, also reviewed, Clementi et al., in press). In Alzheimer's disease, loss of cortical epibatidine binding is related to decreased synaptophysin immunoreactivity (Sabbagh et al., 1998). Immunochemical evidence (Martin-Ruiz et al., 1999) indicates that the loss of cortical receptor binding in Alzheimer's disease reflects changes in α_4 but not α_3 or α_7 subunits and a preliminary immunohistochemical analysis has identified the loss of both α_4 and β_2 reactive fibres (Sparks et al., 1998).

The present investigation includes alterations in nicotinic receptors in Alzheimer's disease examined in relation to clinical and pathological indices of disease severity and in view of an emerging strong correlation with acetylcholinesterase, also nicotinic receptor changes in a transgenic mouse model over expressing acetylcholinesterase (Beeri et al., 1995, 1997). Comparisons are made between Parkinson's disease and Dementia with Lewy bodies and evidence of an early involvement of nicotinic receptors in substantia nigra dopaminergic neuronal degeneration are examined. Potentially protective effects of nicotinic receptor modulation on pathological features of Alzheimer's disease (cortical plaques and tangles) and Parkinson's disease (substantia nigra neuron loss) are also examined in the brains of normal elderly individuals (in which these features occur to a lesser extent with increasing age) chronically exposed to nicotine (tobacco smokers). The hypothesis that nicotinic receptors are centrally involved in the pathological cascade and clinical symptomatology in both Alzheimer and Lewy body diseases is thus explored.

2. Materials and Methods

2.1. Cases

Newcastle series. Cases of Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies, together with age matched controls, were selected according to previously published clinical and pathological diagnostic criteria (Perry et al., 1990; Perry et al., 1995; McKeith et al., 1996; Martin-Ruiz et al., 1999). Also included is a series of middle aged to elderly normal individuals with an established history of tobacco use (aged-matched smokers and non-smokers), described in detail elsewhere (Court et al., 1998).

Mount Sinai, New York series. A full description of the demographic details and neuropathological diagnosis in 81 subjects with clinical dementia rating scales ranging from 0 to 5 is provided by Davis et al. (1999).

2.2. Tissue

Tissue from both series was snap frozen from coronal slices of the left hemisphere and maintained at -70°C, and samples from New York transported on solid CO_2. Brains from the acetylcholinesterase transgenic mice (Beeri et al., 1995, 1997) were frozen at sacrifice, transported on solid CO_2 and stored at -70°C.

2.3. Neurochemical analysis

Nicotinic receptors and acetylcholinesterase activities were determined in hippocampus, entorhinal cortex (Brodmann area BA 28), temporal cortex (BA 36 and 22), parietal cortex (BA 36/40) and putamen immediately rostral of the anterior commissure.

Nicotinic receptor binding was determined in washed membrane preparations expressed in terms of unit protein (Lowry et al., 1951) (Figs. 4, 5 and 7) or autoradiographically (Figs. 1–3 and 6) using [^{3}H]-nicotine, [^{3}H]-epibatidine or [^{125}I]-α-bungarotoxin as previously described (Perry et al., 1990; Houghtling et al., 1995; Court et al., 1997; Martin-Ruiz et al., 1999). Non-specific binding was $< 10\%$ for nicotine and epibatidine, and on average 50% for α-bungarotoxin.

Alpha 4 subunit immunoreactivity was estimated in tissue extracted as previously described (Martin-Ruiz et al., 1999), subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis, with protein transfer by electroblotting onto polyvinyldifluoride membranes which were then processed for immunoreactivity using a monoclonal antibody (NR $\alpha 4$–1.5) raised against the extracellular domain of a human $\alpha 4$ recombinant peptide (at the Helenic Pasteur Institute) and a polyclonal antibody (SC-1772) raised against a carboxy terminus, rat epitope, supplied by Santa Cruz Biotechnology. Amyloid-ß 1–42 levels were determined biochemically (Kaplan et al., 1999).

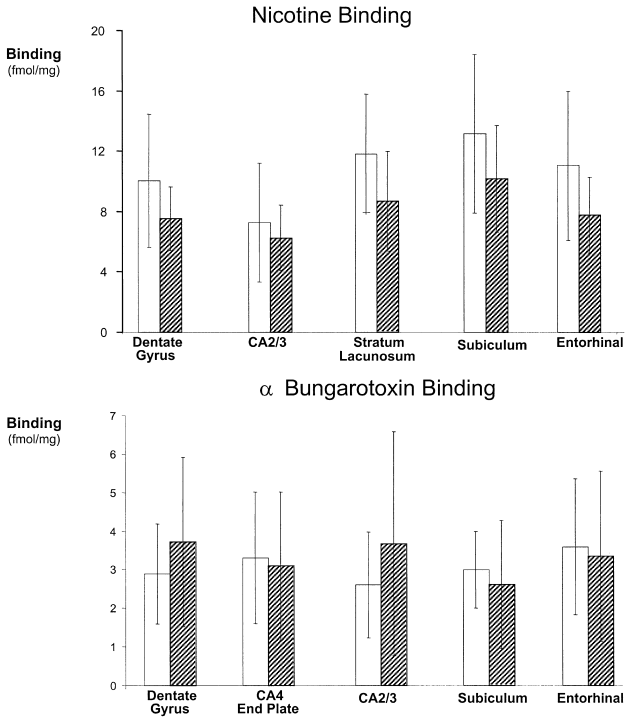

Fig. 1. [^{3}H]-Nicotine and [^{125}I]-α ungarotoxin binding in 13 control (light shading) and 13 Alzheimer (dark shading) cases. Columns represent mean values and bars represent standard deviations, with significant differences ($P < 0.05$) for only nicotine binding in striatum lacunosum and entorhinal cortex. Groups were matched for age (76 ± 9, 80 ± 5 years) and postmortem delay (29 ± 14, 31 ± 18 h).

Amyloid-ß levels were measured in formic acid and extracts of 20–25 mg flash-frozen cortical tissue by a fluorescence-based sandwich enzymatic-linked immuno specific assay using amyloid-ß40 and amyloid-ß42 end-specify polyclonal antibodies. The 4G8 antibody was used as the capture antibody. Characterisation of the antibodies and the specific extraction and enzymatic linked immuno specific assay methods are described in detail by Näslund et al. (in press).

Acetylcholinesterase activity was estimated biochemically in tissue homogenates from the New York series (Davis et al., 1999) and histochemically (Perry et al., 1992) in the transgenic mice brains sectioned sagitally through the left hemisphere, with the inclusion of ethopropazine (10^{-4}M, to inhibit butyrylcholinesterase) in the incubation medium.

Acetylcholinesterase histochemical intensity (which in snap frozen, post-fixed tissue represents the insoluble, membrane-bound compartment of total enzyme activity) was quantified using a Lynx Image Analysis system (Applied Imaging) against a standard grey scale.

2.4. Neuropathological assessments

In addition to diagnostic neuropathology (see above), densities of neocortical senile plaques and of numbers of pigmented (dopaminergic) neurons in the substantia nigra pars compacta were quantified as previously described (Perry et al., 1990). Plaque and tangle distributions and densities in the hippocampus and entorhinal cortex were also assessed on the basis of immunoreactivities using antibodies against amyloid-β (DAKO, clone 6F/3D raised against the synthetic peptide, residues 8–17 with additional C-terminal cysteine residue), and against the phosphorylated Ser 202 residue of tau protein (clone AT8 from Innogenetics). Braak staging (Braak and Braak, 1991) and a modification of this based on Aβ-positive plaque density only (ranging from no plaques in any areas, a few in entorhinal cortex, subiculum clouds, to dentate gyrus and CA1–4) were assessed in the hippocampus and entorhinal cortex or parahippocampal gyrus.

2.5. Statistics

Differences between groups were analysed by one way analysis of variance (ANOVA) and Mann Whitney; correlations by Spearman Rank.

3. Results

3.1. Alzheimer's disease

Fig. 1 illustrates levels of nicotine and α-bungarotoxin binding in regions of the hippocampus and entorhinal cortex in age matched Alzheimer's disease and control cases from the Newcastle series. Nicotine binding was reduced to some extent in all areas, and significantly in stratum lacunosum and entorhinal cortex. In contrast, there were no significant differences in α-bungarotoxin binding between Alzheimer's disease and controls. Although there appeared in this same series to be a relation between increasing density of amyloid-β positive plaques and re-

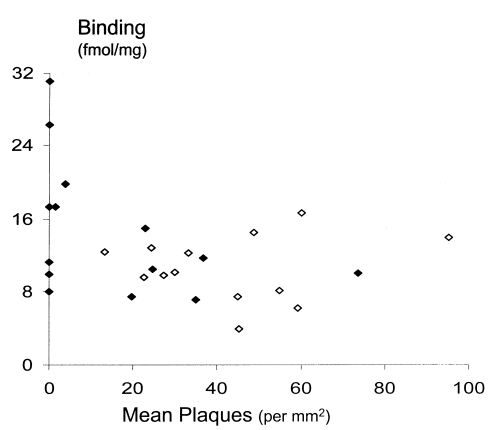

Fig. 2. Relationship between [^{3}H]-nicotine binding and density of amyloid-β-positive plaques in entorhinal cortex. Although the correlation was significant in the combined control and Alzheimer (open symbol) groups ($r = 0.48$, $P < 0.01$), this did not reach significance for either group alone ($r = -0.26$, $r = 0.11$).

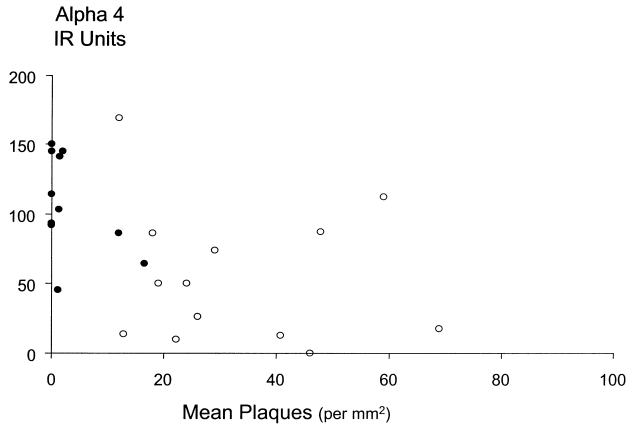

Fig. 3. Relationship between α4 immunoreactivity and plaque densities in the temporal neocortex, with no significant correlation within the control or Alzheimer (open symbol) groups ($r = -0.54$, $r = -0.16$) despite a significant correlation in the combined groups ($r = -0.51$, $P < 0.01$).

duced nicotine binding in entorhinal cortex (Fig. 2), this was only significant if both control and Alzheimer's disease groups were combined, but not in either group alone. In the temporal cortex, a similar situation was apparent for the relation between increasing plaque densities and loss of α4 subunit immunoreactivity (Fig. 3). There was a significant inverse correlation in temporal cortex (Mount Sinai, New York series) between levels of Amyloid-β1–42 (but not amyloid-β1–40) and loss of epibatidine binding (Fig. 4), the latter again not significantly related to plaque density, nor to neurofibrillary tangle formation. In contrast a significant positive correlation existed between plaque densities and α-bungarotoxin binding in entorhinal cortex which applied to both the Alzheimer's disease and control groups analysed separately or together (Fig. 5).

In the New York series, epibatidine binding in temporal cortex (BA 22 and 36) was also inversely correlated with the clinical dementia rating (CDR) ($r = -0.302$ and

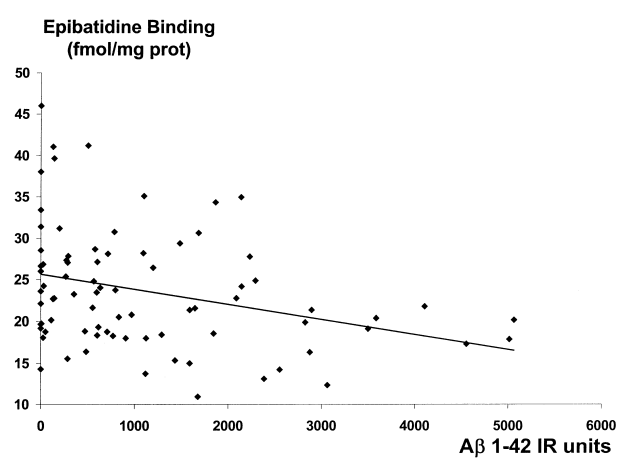

Fig. 4. Relationship between [^{3}H]-epibatidine binding and levels of amyloid-β1–42 in individuals (CRD 0–5) from the Mount Sinai series, $r = -0.315$, $P = 0.004$.

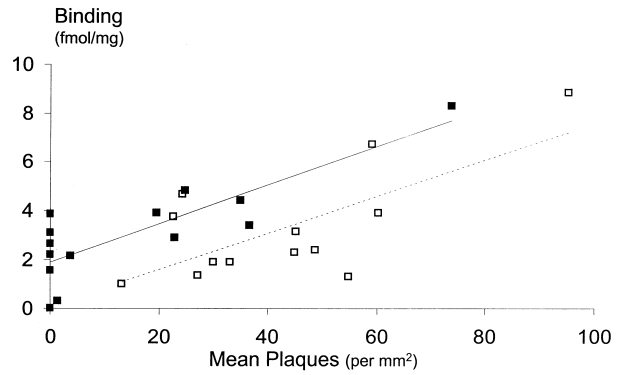

Fig. 5. Positive correlation between [^{125}I]-α bungarotoxin binding and Aβ-positive plaque densities in entorhinal cortex ($r = 0.84$, $P < 0.001$, $r = 0.70$, $P < 0.01$ and $r = 0.65$, $P < 0.02$ in the control, Alzheimer (open symbol), and combined groups, respectively).

-0.312, $P < 0.01$) with evidence (Fig. 6) of small decrements in receptor binding in the groups with mildest cognitive impairment (CDR 0.5 vs. CDR O). Amongst all parameters investigated in relation to epibatidine binding in this series, the strongest correlation was found with acetylcholinesterase activity ($r = 0.52$, $P < 0.001$). In

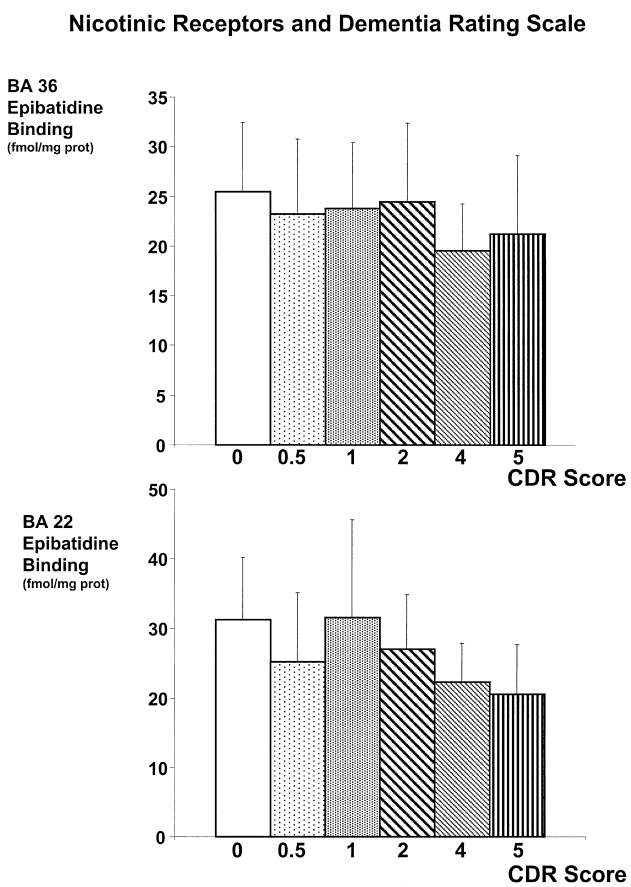

Fig. 6. Epibatidine binding in temporal cortex from the Mount Sinai series, grouped according to severity of dementia (CDR). There were significant inverse correlations in both Brodmann areas 36 (above) and 22 (below) ($r = -0.30$, $r = -0.31$, $P < 0.01$).

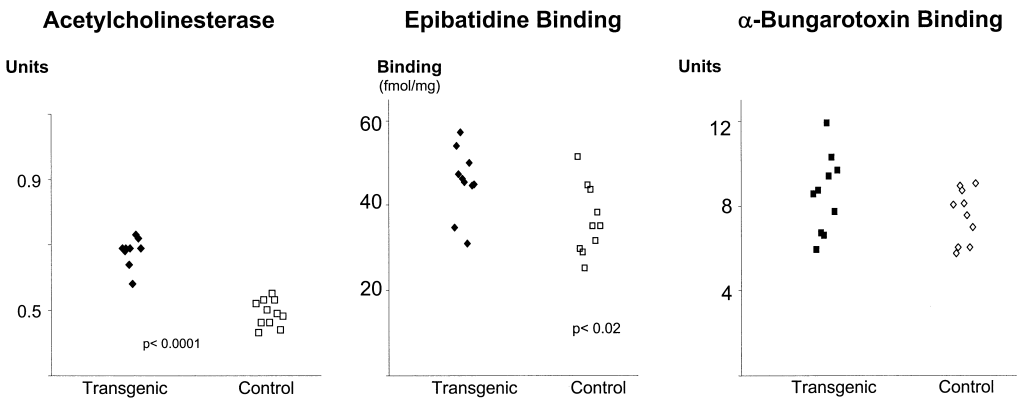

Fig. 7. Comparison between the acetylcholinesterase transgenic and age-matched control mice brains. These were in the transgenic (open symbol) group significantly elevated acetylcholinesterase ($P < 0.0001$), and epibatidine binding ($P < 0.02$) but not α bungarotoxin binding the cerebral cortex.

Brodmann area 22 this correlation persisted and in fact grew more significant ($r = 0.67$, $n = 38$, $P < 0.001$) when only the subjects meeting the neuropathological diagnostic criteria for Alzheimer's disease were included, and the correlation remained when only those subjects who met neuropathological (CERAD) criteria for normal were considered ($r = 0.42$, $n = 25$, $P = 0.037$). This finding led to the analysis of nicotinic receptors in the transgenic mouse model over-expressing acetylcholinesterase (Beeri et al, 1995, 1997) to explore further interactions between the receptor and enzyme.

3.2. Acetylcholinesterase transgenic mice

Fig. 7 illustrates that in conjunction with elevated acetylcholinesterase activity, there is in the cerebral cortex a significant elevation in epibatidine binding in the transgenic group. There was a similar, but not significant trend, for α-bungarotoxin binding. In the striatum (data not shown), similar changes in nicotinic receptor binding were observed which were significant for both epibatidine and α-bungarotoxin binding.

3.3. Lewy body disorders

In comparison with Alzheimer's disease, nicotine binding is in parietal cortex as, if not more, extensively reduced in dementia with Lewy bodies, whereas in dorsal putamen significant reductions are apparent in dementia with Lewy bodies and Parkinson's disease but not Alzheimer's disease (Fig. 8). The loss in dementia with Lewy bodies is nearly as extensive as in Parkinson's disease, a finding that is compatible with equally extensive

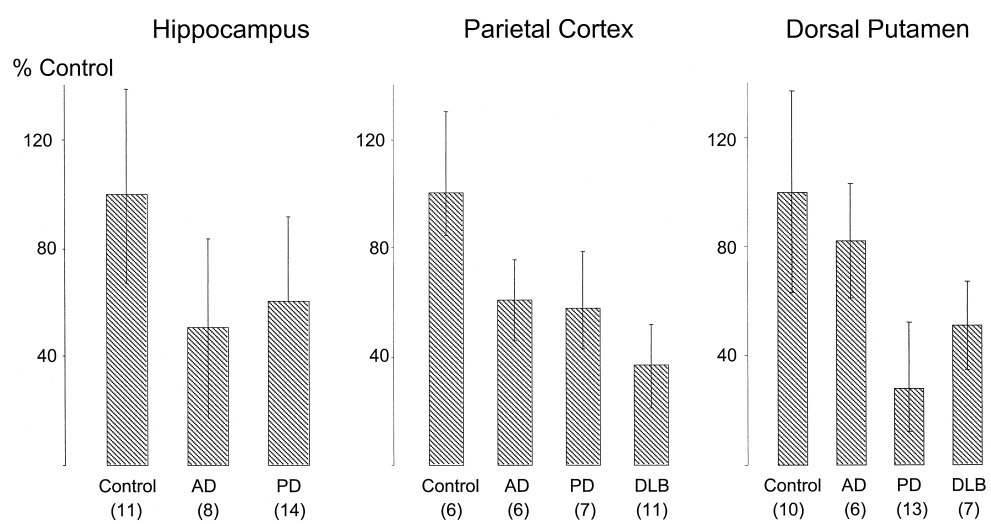

Fig. 8. [^{3}H]-Nicotine binding as a percentage of control in different regions of cases of age-matched Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Reductions were compared to control significant ($P < 0.05 - P < 0.001$) in all groups and areas except putamen in AD. Data taken from Perry et al, 1990, 1995 (cortex and hippocampus) and Court et al., submitted (dorsal putamen). In the putamen series controls were non-smokers and AD and DLB cases were non-neuroleptic treated. Case numbers in parentheses.

Table 1
Spread and severity of neurofibrillary tangle (AT8) and amyloid-β plaque formation in hippocampus and entorhinal cortex in relation to tobacco smoking history

	Non-smokers	Smokers
Braak stages		
0	5	6
1	0	2
2	2	2
3	3	6
4	6	4
Plaque 'stages'*		
0	1	9
1	2	3
2	6	0
3	4	3
4	3	0

*Mean plaque stage (2.4 ± 1.1, 0.8 ± 1.2) significantly different between the two groups ($P = 0.0026$ Mann–Whitney U). Mean ages were 82 ± 2 and 73 ± 4 years.

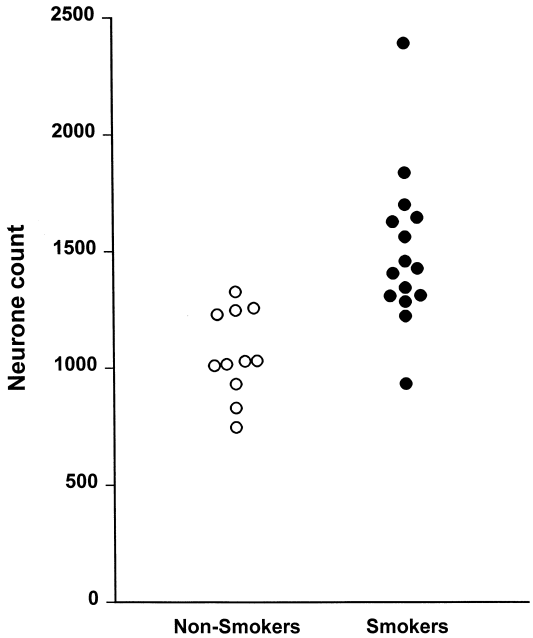

Fig. 10. Substantia nigra pars compacta pigmented neuron number in individual non-smokers and smokers (all female, groups age-matched-83 ± 12, 76 ± 5 yr).

reductions in nicotine binding in the substantia nigra, despite much greater substantia nigra loss in Parkinson's disease (Perry et al., 1995). In contrast to the loss of cortical nicotine binding in Alzheimer's disease (above), there was, despite a reduction in striatal nicotine binding, no change in the immunoreactivity of the α4 subunit in the striatum in Parkinson's disease (mean 104.8 ± 51.8 and 139.8 ± 55.3 IR units in 9 control and 9 Parkinson's disease cases), nor in a preliminary analysis of the α3 or α6 (Martin-Ruiz et al., submitted).

3.4. Chronic nicotine exposure and normal human brain ageing

Tobacco exposure is associated with an increase (37%) in α4 but not α3 immunoreactivity in temporal cortex (Martin-Ruiz et al., 1999). Comparing the hippocampus

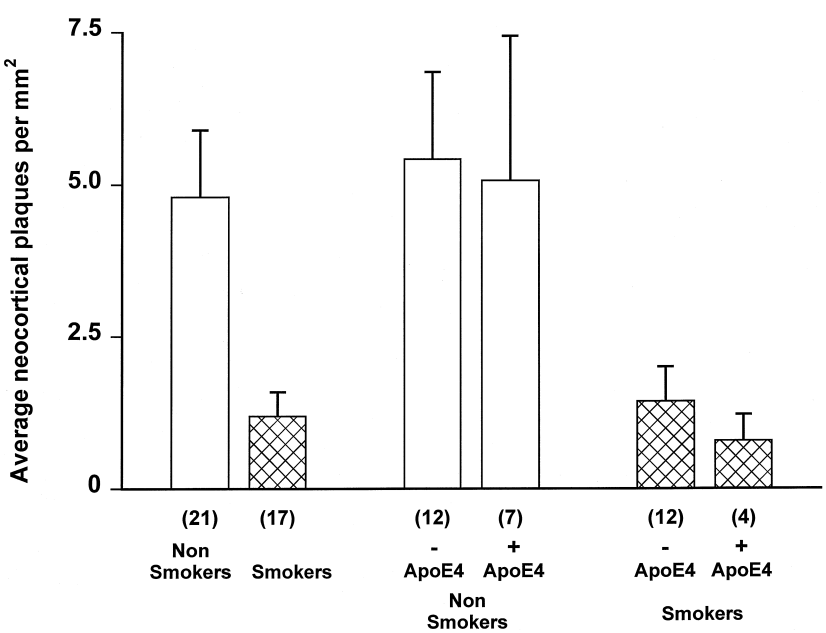

Fig. 9. Mean neocortical plaque densities in normal aged individuals who were and were non-exposed to chronic nicotine (tobacco smokers compared to non-smokers). The middle and right hand column sets contain subgroups according to apolipoprotein E ε4 genotype. Columns represent mean values and bars represent standard deviations with case numbers in parentheses. Differences between non-smokers and smokers reached significance for the whole group ($P = 0.036$) and for the Apo E4-negative subgroups ($P = 0.02$). Groups were matched for age (non-smokers 83 ± 6, smokers 79 ± 7 years).

and entorhinal cortex neuropathologically from age matched normal individuals who were exposed to tobacco smoke within 10 yr of death with those who were either non-smokers or had ceased over 10 yr before death, the severity of tangle formation (Braak staging) was similar in both groups, but the extent of plaque formation was significantly lower in the smoking group (Table 1). Plaque formation in the neocortex was also significantly lower in smokers compared with age-matched non-smokers (Fig. 9), a difference that was independent of apolipoprotein E genotype.

The density of pigmented substantia nigra pars compacta neurons was also significantly different between the two groups, being higher in the smokers (Fig. 10).

4. DISCUSSION

In relation to the questions posed in the Section 1, the data described support the following conclusions:

1. Although there is a generalised loss of different nicotinic receptors subtypes in aging, nicotinic receptor loss in the cortex in Alzheimer's disease selectively involves the subtype containing the $\alpha 4$ subunit, but not α_3 or α_7, whereas the striatal receptor loss in Parkinson's disease reflects a subunit other than α_3, α_4 or α_6.

2. The receptor loss in Alzheimer's disease is more closely associated with increased levels of the amyloid-$\beta 1-42$ peptide than with senile plaque formation and there is no relation to tangle formation; this suggests an involvement of the α_4 subunit in the earlier as opposed to later stages of Alzheimer type pathology.

3. The loss of nicotinic receptors from dopaminergic neurons and their projections to striatum is likely to be an early change in Lewy body disease, since nicotine binding is extensively reduced in both Parkinson's disease and dementia with Lewy bodies, the latter being affected by less neuron loss.

4. Chronic nicotine exposure is associated in the normal aged human brain with attenuation of senile plaque formation and substantia nigra neuron loss, evidence consistent with neuroprotective effects of nicotinic agonists in experimental cell or animal models (reviewed Clementi et al., 1999) and epidemiological evidence, particularly on Parkinson's disease (Ben Schlomo, 1997; Gorell et al., 1999).

5. Reduced nicotinic receptor binding is related to dementia severity, assessed using a global rating, although more detailed clinical correlates are needed (e.g. in relation to symptoms such as attentional deficits, nociception, conscious awareness, motor dysfunction and depression). The greater reduction of parietal cortex nicotine binding in dementia with Lewy bodies compared to Alzheimer's disease may, for example, relate to greater visuospatial deficits in dementia with Lewy bodies.

The strong correlation between loss of high affinity nicotinic agonist binding and acetylcholinesterase raises new questions about the cascade of events associated with cholinergic dysfunction. The experimental data on the acetylcholinesterase transgenic mice supports the concept of a mechanism of acetylcholinesterase-nicotinic receptor co-regulation, which needs to be examined further in terms of translational or post-translational interactions, and in nicotinic receptor subunit knockout models.

In relation to one of the most exciting areas of nicotinic receptor research — neuroprotection in the ageing brain, these collective data suggest that the $\alpha 4$ subunit is intimately involved in the earliest stages of cortical β-amyloidosis, but that the subunit associated with nigrostriatal pathology remains to be determined.

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References

Beeri, R., Andres, C., Lev-Lehman, E., Timberg, R., Huberman, T., Shani, M., Soreq, H., 1995. Transgenic expression of human acetylcholinesterase induces progressive cognitive deterioration in mice. Curr. Biol. 5, 1063–1071.

Beeri, R., LeNovere, N., Mervis, R., Huberman, T., Grauer, E., Changeux, J.P., Soreq, H., 1997. Enhanced hemicholinium binding and attenuated dendrite branching in cognitively impaired acetylcholinesterase-transgenic mice. J. Neurochem. 69, 2441–2451.

Ben-Shlomo, Y., 1997. The epidemiology of Parkinson's disease. Baillieres Clin. Neurol. 6, 55–68.

Braak, H., Braak, E., 1991. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 82, 239–259.

Clementi, F., Court, J.A., Perry, E., 1999. Involvement of neuronal nicotinic receptors in disease. In: Clementi, F., Fornasari, D., Gotti, C. (Eds.), Handbook of Experimental Pharmacology. Springer, Heidelberg.

Court, J.A., Lloyd, S., Johnson, M., Griffiths, M., Birdsall, N.J.M., Piggott, M.A., Oakley, A.E., Ince, P.G., Perry, E.K., Perry, R.H., 1997. Nicotinic and muscarinic cholinergic receptor binding in the human hippocampal formation during development and aging. Dev. Brain Res. 101, 93–105.

Court, J.A., Lloyd, S., Thomas, N., Piggott, M.A., Marshall, E.F., Morris, C.M., Lamb, H., Perry, R.H., Johnson, M., Perry, E.K., 1998. Dopamine and nicotinic receptor binding and the levels of dopamine and homovanillic acid in human brain related to tobacco use. Neuroscience 87, 63–78.

Court, J.A., Piggott, M.A., Perry, E.K., Barlow, R.B., Perry, R.H., 1992. Age associated decline in high affinity nicotine binding in human frontal cortex does not correlate with changes in choline acetyltransferase activity. Neurosci. Res. Commun. 10, 125–133.

Davis, K.L., Mohs, R.C., Marin, D., Purohit, D.P., Lantz, M., Austin, G., Haroutunian, V., 1999. Cholinergic markers in elderly patients with early signs of Alzheimer's disease. J. Am. Med. Assoc. 281, 1401–1406.

Flynn, D.D., Mash, D.C., 1986. Characterization of L-[^{3}H]nicotine binding in human cerebral cortex: comparison between Alzheimer's disease and the normal. J. Neurochem. 47, 1948–1954.

Gorell, J.M., Rybicki, B.A., Johnson, C.C., Peterson, E.L., 1999. Smoking and Parkinson's disease: a dose response relationship. Neurology 52, 115–119.

Hellström-Lindahl, E., Court, J.A. Nicotinic acetylcholine receptors during prenatal development and brain pathology in human aging. Behav. Brain Res., in press.

Houghtling, R.A., Davila-Garcia, M.I., Kellar, K.J., 1995. Characterization of (+/−)(−)[^{3}H]epibatidine binding to nicotinic cholinergic receptors in the rat and human brain. Mol. Pharmacol. 48, 280–287.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 153, 265–275.

McKeith, I.G., Galasko, D., Kosaka, K., Perry, E.K., Dickson, D.W., Hansen, L.A., Salmon, D.P., Lowe, J., Mirra, S.S., Byrne, E.J., Lennox, G., Quinn, N.P., Edwardson, J.A., Ince, P.G., Bergeron, C., Burns, A., Miller, B.L., Lovestone, S., Collerton, D., Jansen, E.N., Ballard, C., de Vos, R.A., Wilcock, G.K., Jellinger, K.A., Perry, R.H., 1996. Dementia with Lewy bodies: diagnostic criteria and pathological guidelines. Report of the consortium on dementia with Lewy bodies. Neurology 47, 1113–1124.

Martin-Ruiz, C.M., Court, J.A., Molnar, E., Lee, M., Gotti, C., Mamalaki, A., Tsouloufis, T., Tzartos, S., Ballard, C., Perry, R.H., Perry, E.K., 1999. α4 but not α3 and α7 nicotinic acetylcholine receptor subunits are lost from the temporal cortex in Alzheimer's disease. J. Neurochem. 73, 1635–1640.

Näslund, J., Haroutunian, V., Mohs, R., Davis, K.L., Davies, P., Greengard, P., Buxbaum, J.D., in press. Elevated amyloid β peptides in brain: correlation with cognitive decline, JAMA.

Nordberg, A., Alafuzoff, I., Winblad, B., 1992. Nicotinic and muscarinic subtypes in the human brain: changes with aging and dementia. J. Neurosci. Res. 31, 103–111.

Perry, E.K., Johnson, M., Kerwin, J.M., Piggott, M.A., Court, J.A., Ince, P.G., Shaw, P.J., Brown, A., Perry, R.H., 1992. Convergent cholinergic activities in aging and Alzheimer's disease. Neurobiol. Aging 13, 393–400.

Perry, E.K., Morris, C.M., Court, J.A., Cheng, A., Fairbairn, A.F., McKeith, I.G., Irving, D., Brown, A., Perry, R.H., 1995. Alteration in nicotine binding sites in Parkinson's disease, Lewy body dementia and Alzheimer's disease: possible index of early neuropathology. Neuroscience 64, 385–395.

Perry, E.K., Perry, R.H., Smith, C.J., Purohit, D., Bonham, J., Dick, D.J., Candy, J.M., Edwardson, J.A., Fairbairn, A., 1986. Cholinergic receptors in cognitive disorders. Can. J. Neurol. 13, 521–527.

Perry, R.H., Irving, D., Blessed, G., Fairbairn, A.F., Perry, E.K., 1990. Senile dementia of Lewy body type: a clinically and pathologically distinct form of Lewy body dementia in the elderly. J. Neurol. Sci. 95, 119–139.

Sabbagh, M.N., Reid, R.T., Corey-Bloom, J., Rao, T.S., Hansen, L.A., Alford, M., Masliah, E., Adem, A., Lloyd, G.K., Thal, L.J., 1998. Correlation of nicotinic binding with neurochemical markers in Alzheimer's disease. J. Neural Transm. 105, 709–717.

Schröder, H., Giacogini, E., Struble, R.G., Zilles, K., Maelicke, A., 1991. Nicotine cholinoceptive neurons of the frontal cortex are reduced in Alzheimer's disease. Neurobiol. Aging 12, 259–262.

Sparks, D.L., Beach, T.G., Lukas, R.J., 1998. Immunohistochemical localization of nicotinic β2 and α4 receptor subunits in normal human brain and individuals with Lewy body and Alzheimer's disease: preliminary observations. Neurosci. Lett. 256, 151–154.

Terzano, S., Court, J., Fornasari, D., Griffiths, M., Spurden, D., Lloyd, S., Perry, E., Clementi, F., 1998. Expression of the α3 nicotinic receptor subunit mRNA in human brain in aging and Alzheimer's disease. Mol. Brain. Res. 63, 72–78.

Tohgi, H., Utsugisawa, K., Yoshimura, M., Nagane, Y., Mihara, M., 1998a. Alterations with aging and ischemia in nicotinic acetylcholine receptor subunits α4 and β2 messenger RNA expression in post mortem human putamen. Brain Res. 791, 186–190.

Tohgi, H., Utsugisawa, K., Yoshimura, M., Nagane, Y., Mihara, M., 1998b. Age-related changes in nicotinic acetylcholine receptor subunits alpha4 and beta2 messenger RNA expression in postmortem human frontal cortex and hippocampus. Neurosci. Lett. 245, 139–142.

Utsugisawa, K., Nagane, Y., Tohgi, H., Yoshimura, M., Ohba, H., Genda, Y., 1999. Changes with aging and ischemia in nicotinic acetylcholine receptor subunit α7 mRNA expression in postmortem human frontal cortex and putamen. Neurosci. Lett. 270, 145–148.

Warpman, U., Nordberg, A., 1995. Epibatidine and A-bungarotoxin 418 reveal selective losses of alpha4 beta2 nicotinic receptors in Alzheimer brains. NeuroReport 6, 2419–2423.