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# Nicotinic Acetylcholine Receptor Distribution in Alzheimer's Disease, Dementia with Lewy Bodies, Parkinson's Disease, and Vascular Dementia: *In Vitro* Binding Study Using 5-[$^{125}$I]-A-85380

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Nicotinic acetylcholine receptors (nAChRs) have been implicated in a number of neurological disorders. 5-Iodo-3-[2(*S*)-2-azetidinylmethoxy]pyridine (5-I-A-85380) is a novel nAChR marker, binding predominantly to the $\alpha4\beta2$ subtype. This *in vitro* autoradiography study describes the distribution of 5-[$^{125}$I]-A-85380 binding in post-mortem brain tissue from normal elderly individuals and from cases with age-associated dementias of both neurodegenerative and vascular types. The binding distribution of 5-[$^{125}$I]-A-85380 in normal brain tissue was found to be consistent with the reported distribution of other high-affinity nicotinic ligands. In addition to high thalamic and moderate striatal and temporal cortex density, moderate 5-[$^{125}$I]-A-85380 binding was also seen in white matter tracts in cingulate, occipital, and temporal areas, indicating the presence of nAChRs along nerve fiber tracts, which has not been reported in other high-affinity nicotinic agonist distribution studies. In Parkinson's disease (PD), loss of striatal 5-[$^{125}$I]-A-85380 binding closely parallels the loss of nigrostriatal dopaminergic markers previously observed. In dementia with Lewy bodies (DLB) reduced striatal 5-[$^{125}$I]-A-85380 binding density, comparable to that in PD, may be a marker of early degeneration in nigrostriatal inputs, while in Alzheimer's disease (AD) reduced striatal 5-[$^{125}$I]-A-85380 binding could be related to reduced cortical inputs. The reductions of nAChRs seen in AD, DLB, and PD were not apparent in vascular dementia (VaD). In conclusion, 5-I-A-85380 is clearly a useful ligand for both *in vitro* and *in vivo* single photon emission tomography human studies investigating disease symptoms and progression, response to acetylcholinesterase-inhibiting drugs and in differentiating primary degenerative dementia from VaD.
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**Keywords:** nicotinic receptor; 5-I-A-85380; Alzheimer's disease; dementia with lewy bodies; Parkinson's disease; vascular dementia

## INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that are widely distributed in the human brain where they have a modulatory function associated with numerous transmitter systems. The major nAChR subtypes present in the mammalian brain are $\alpha7$ and $\alpha4\beta2$ (Lindstrom *et al*, 1995; Holladay *et al*, 1997). The majority of brain nAChRs with a high affinity for agonists contain $\alpha4$ and $\beta2$ subunits (Picciotto *et al*, 1995; Marubio *et al*, 1999). [$^3$H]cytisine and [$^3$H]ABT-418 predominantly bind to $\alpha4\beta2$, whereas [$^3$H]epibatidine and [$^3$H]nicotine bind in addition to $\alpha3$ and possibly other subunit-containing receptors (Flores *et al*, 1992; Sihver *et al*, 1998; Zoli *et al*, 1998).

Reductions in nAChR density have been identified in a number of neurodegenerative disorders including Alzheimer's disease (AD), dementia with Lewy bodies (DLB), and Parkinson's disease (PD) (Rinne *et al*, 1991; Perry *et al*, 1995; Court *et al*, 2000a). In AD, binding assays with [$^3$H]ABT-418 and [$^3$H]epibatidine (Warpman and Nordberg, 1995) and immunochemical analysis (Martin-Ruiz *et al*, 1999; Wevers *et al*, 1999; Guan *et al*, 2000; Burghaus *et al*, 2000) indicate that the major contributor to loss of nAChRs in cortical areas is the $\alpha4$ subunit. Deficits in [$^{11}$C]nicotine binding in the hippocampus, temporal and frontal cortex have also been reported *in vivo* in AD (Paterson and Nordberg, 2000). In PD, the majority of

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reports indicate deficits in high-affinity nicotinic agonist binding in both the caudate and putamen (Perry *et al*, 1990a; Rinne *et al*, 1991; Aubert *et al*, 1992a; Court *et al*, 2000b), in parallel with the decline in dopaminergic markers (Piggott *et al*, 1999). Reduced [$^3$H]nicotine binding in the substantia nigra (SN) pars compacta and ventral tegmental area has also been reported in PD (Perry *et al*, 1995). In DLB, losses in [$^3$H]nicotine binding have been reported in the striatum, the SN pars compacta and pars reticulata, and the dorsolateral tegmentum (Perry *et al*, 1995; Court *et al*, 2000b).

5-Iodo-3-[2(*S*)-2-azetidinylmethoxy]pyridine (5-I-A-85380) is a high-affinity nAChR ligand that demonstrates high subtype selectivity for $\alpha4\beta2$ nAChRs (Mukhin *et al*, 2000). A recent study has also shown 5-I-A-85380 binding to $\alpha$-conotoxin MII-sensitive ($\alpha6\beta2$) nAChRs in the rodent and monkey striatum (Kulak *et al*, 2002). The development of an *in vivo* $\alpha4\beta2$ subtype-selective nAChR imaging agent will allow studies following disease progression, identifying where and when during the disease process nAChR deficits occur, and also provide information on how nAChR's expression relates to symptomatology and therapeutic response.

The present study investigates the distribution of 5-[$^{125}$I]-A-85380 binding in the midbrain, neocortex, and cerebellum in normal elderly brain autopsy tissue by autoradiography. In addition, comparison of 5-[$^{125}$I]-A-85380 binding was made between cases of AD, mild AD, DLB, PD, vascular dementia (VaD), and normal elderly aged-matched controls. The study aims to provide reference data for use in clinical research SPECT neuroimaging studies using 5-[$^{123}$I]-A-85380.

## MATERIALS AND METHODS

### Materials

The trimethylstannyl precursor (TMS precursor) of 5-[$^{125}$I]-A-85380 and cold 5-I-A-85380 was obtained from ABX, Germany. Chemicals used in radiolabelling and in autoradiography assays were obtained from Sigma Aldrich, UK and Vectabond was from Vector Labs, UK. Na$^{125}$I and $^{125}$I autoradiographic microscale standards were obtained from Amersham Pharmacia Biotech UK Ltd, UK. High-performance liquid chromatography (HPLC) solvents were obtained from Rathburn Chemicals Ltd, UK and HPLC columns were obtained from Phenomenex, UK. Autoradiography film was obtained from Perkin-Elmer, Belgium and developer and fix was obtained from Kodak, HA West, UK.

### Radiochemistry

5-[$^{125}$I]-A-85380 was synthesized via a modification of a previously published method by Zoghbi *et al* (2001). Aliquots (300 μg) of TMS precursor were stored in nitrogen-filled, silanized vials at −18°C. A measure of 74 MBq, carrier free, Na$^{125}$I in ~18 μl of 0.01–0.1 M NaOH was obtained and was made up to 200 μl using 0.01 M NaOH. The radioiodination reaction was prepared by adding 20 μl 2 M HCl to the activity followed by addition of a mixed solution of 300 μg of precursor in 100 μl ethanol. The reaction was initiated by adding 50 μl (50 μg) of Chloramine T and the reaction mixture was then mixed by vortex. Mixing was continued during an incubation period of 15 min at 65°C. The reaction was quenched by addition of 100 μl (100 μg) $NaHSO_4$. Deprotection was achieved by addition of 200 μl of trifluoroacetic acid and mixing. Mixing was continued during an incubation period of 15 min at 65°C.

Purification of 5-[$^{125}$I]-A-85380 was carried out by injection of the reaction mixture onto a reverse phase C-18 semi-preparative column (Synergi Hydro 4 μm 10 × 150 mm) at a flow rate of 3 ml/min. The fraction containing 5-[$^{125}$I]-A-85380 was collected and the solvent removed by rotatory evaporation. Reconstitution of the purified 5-[$^{125}$I]-A-85380 was carried out with sterile 0.9% saline solution through a 0.22 μm filter. The 5-[$^{125}$I]-A-85380 obtained was then stored at −20°C. Analytical HPLC of an aliquot of the final product was carried out on a reverse phase C-18 analytical column (Synergi Hydro 4 μm, 4.6 × 150 mm) at a flow rate of 1 ml/min.

### Stability of 5-[$^{125}$I]-A-85380

The stability of 5-[$^{125}$I]-A-85380 in saline was investigated over time at −20°C. Samples were stored and analyzed at $5\frac{1}{2}$, 9, and 13 weeks. Samples were injected on to a reverse phase C-18 analytical column (Synergi Hydro 4 μm, 4.6 × 150 mm) at a flow rate of 1 ml/min.

### Brain Tissue

Joint Ethics Committee of Newcastle and North Tyneside Health Authority rules and MRC brain banking procedures were followed and consent of next of kin was obtained in the collection of all brain tissue. Coronal slices from the left hemisphere were obtained postautopsy and were rapidly frozen in liquid arcton cooled over liquid nitrogen. The slices were stored at −70°C.

For the distribution study, tissue from 16 control cases was used. Demographic details of control cases are shown in Table 1. Smoking histories were obtained, one smoker was excluded from the study and ex-smokers identified. The control group studied had not been exposed to neuroleptic medication. Tissue blocks were obtained containing striatum, globus pallidus, insula and cingulate cortices as well as blocks of anterior thalamus, temporal cortex, occipital cortex, frontal cortex, and cerebellum. Not all areas studied were available in every case.

For the disease study, tissue from 16 controls cases, 24 DLB cases, 8 AD cases, 4 mild AD cases, 10 VaD cases, and 10 PD cases was used. Demographic details of cases are shown in Table 1. No significant differences in age were seen between groups. Smoking histories were obtained for all groups, one smoker was excluded from the study and ex-smokers identified. Neuroleptic drug histories were also identified. None of the cases used in this study were exposed to cholinesterase inhibitors. Tissue blocks were obtained containing striatum, globus pallidus, insula and cingulate cortices as well as blocks of anterior thalamus and temporal cortex. The majority of areas were obtained from most cases.

**Table 1** Case Demographic Details for Disease Study with 5-[$^{125}$I]-A-85380

| Group (*n*) | Age (years) | Gender | PM delay (hours) | Ex-smokers (*n*) |
|---|---|---|---|---|
| Control (16) | 83.1 ± 10.7 | 11F/5M | 34.0 ± 18.5 | (5) |
| DLB (24) | 78.6 ± 6.4 | 12F/12M | 37.6 ± 23.2 | (5) |
| AD (8) | 80.6 ± 8.0 | 3F/5M | 33.9 ± 26.2 | (2) |
| Mild AD (4) | 80.5 ± 4.8 | 3F/1M | 65.8 ± 16.9 | (1) |
| VaD (10) | 81.7 ± 9.7 | 7F/3M | 45.8 ± 24.5 | (1) |
| PD (10) | 78.1 ± 8.6 | 5F/5M | 46.1 ± 28.3 | (0) |

There are no significant differences between the groups except for a significantly longer post-mortem (PM) delay in the mild AD group (one-way ANOVA). PM delay in the mild AD group does not correlate with 5-[$^{125}$I]-A-85380 binding in any areas measured. The known smoker has been eliminated and ex-smokers have been identified in these data. *n* = number of cases.

For autoradiography, sections were cut by cryostat (20 μm) and dried on to Vecta-bond-coated slides at room temperature before storage in sealed boxes at −70°C.

### Neuropathological Diagnosis

At post-mortem, the right hemisphere was formalin fixed for at least 3 months. AD pathology was evaluated using quantitative techniques to determine plaque and tangle densities (Perry *et al*, 1990b). Sections of cortical areas were stained with a modification of Palmgren's silver technique for neurofibrillary tangle quantification (Cross, 1982). The von Braunmühl silver impregnation technique was used to demonstrate plaques. For each neocortical area, the mean tangle density was obtained in consecutive fields (0.61 mm$^2$/area) through the full width of the cortical ribbon in five randomly marked positions around gyri; the mean plaque density was calculated from counts in fields (3.1 mm$^2$/area) at five similarly marked points. Tangle and plaque densities in the whole of the neocortex were expressed as mean values per mm$^2$. Braak staging was determined (Braak and Braak, 1991) and AD was defined as Braak stage V and VI. Mild AD was defined as cases with no clinical confirmation of dementia but demonstrating senile plaque densities diagnostic for possible AD and neurofibrillary changes characteristic of early AD (Braak stage ⩽III). α-Synuclein immunohistochemistry (Novocastra Laboratories Ltd, Newcastle, UK) was used to demonstrate Lewy bodies (LB) and Lewy neurites. Cortical LB frequency was assessed according to the consensus guidelines for diagnostic rating protocol of DLB (McKeith *et al*, 1996), which have been modified for α-synuclein, an LB pathology marker more sensitive than the earlier recommended ubiquitin. Scoring for each cortical area for α-synuclein was: 0 = no LB, 1 = up to five LB, 2 = 5–20 LB plus few neurites, 3 = 20–40 LB plus moderate neurites, 4 = over 40 LB plus dense neurites. Controls had no evidence of neurological disease. Controls were assessed neuropathologically, and did not show AD pathology or LB commensurate with a diagnosis of AD or other neurodegenerative disease. No significant vascular pathology was seen in controls.

### Autoradiography Procedures

Prior to assay, sections were left to air dry at room temperature. Adjacent sections were prewashed in Tris-HCl pH 7.4 buffer, containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1 mM $MgCl_2$, for 20 min at room temperature to remove endogenous ligand. For total binding, sections were incubated in the same buffer as prewash and 22 pM 5-[$^{125}$I]-A-85380 for 4 h at room temperature. The Kd of 5-[$^{125}$I]-A-85380 has been reported to be 12 pM in the human brain (Mukhin *et al*, 2000); therefore, at the above concentration binding approaches $B_{max}$. A measure of 1 μM epibatidine was added to at least one adjacent section from each case to give a measure of nonspecific binding. After incubation, sections were washed for 2 × 10 min in the same buffer at 4°C and dipped into distilled water at 4°C. Sections were then left to air dry for 1 h before exposure to Kodak Biomax MR film (24 × 30 cm) with $^{125}$I autoradiographic microscale standards at 4°C. After 48 h exposure, the films were warmed to room temperature (2 h) before development using 500 ml D19 for 5 min, stopped using 500 ml 1% aqueous acetic acid for 1 min, fixed using 500 ml 25% Unifix for 6 min, and washed for 20 min in running water. Films were left to air dry for 2 h. Binding was quantified to $^{125}$I standards using MCID M5+ image analysis system (Imaging Research Ltd, Interfocus, UK) to give binding in fmol/mg of tissue. Specific binding was calculated by subtraction of non-specific binding from mean total binding.

### Statistical Analysis

Binding data in control and disease groups were analyzed by one-way ANOVA with *post hoc* analysis using Fisher's multiple comparison of means. Differences, between groups, in age and post-mortem delay were analyzed by one-way ANOVA with *post hoc* analysis using Fisher's multiple comparison of means, and correlations between binding and demographic factors were analyzed by Pearson's product moment correlation. Binding data in ex-smokers and non-smokers, and neuroleptic exposed and non-exposed, were analyzed by one-way ANOVA with *post hoc* analysis using Fisher's multiple comparison of means. Analysis was carried out using Minitab (Release 13, Minitab Inc.)

## RESULTS

### Radiochemistry

Modification of a previously reported method (Zoghbi *et al*, 2001) has allowed radiolabelling and purification of 5-$^{125}$IA via a more rapid, one-pot synthesis method. 5-[$^{125}$I]-A-85380 was successfully obtained with a radiochemical purity of >98% and an isolated radiochemical yield of 60.2%. The specific activity of the product was calculated to be 2580 Ci/mmol.

### Stability of 5-[$^{125}$I]-A-85380 in Saline

After 9 weeks, the radiochemical purity of 5-[$^{125}$I]-A-85380 stored at −20°C was found to be 96.9% ± 3.0 ($n$ = 2). By 13 weeks, the radiochemical purity of 5-[$^{125}$I]-A-85380 stored at −20°C was reduced to 61.1% ± 2.6 ($n$ = 4). All auto-

radiography assays were completed within 6 weeks after the synthesis of 5-[$^{125}$I]-A-85380.

### Effects of Demographic Variables, Neuroleptic and Smoking Histories

There were no significant correlations in 5-[$^{125}$I]-A-85380 binding with age and post-mortem delay. In addition, no significant differences in 5-[$^{125}$I]-A-85380 binding were found between ex-smokers and non-smokers in all areas studied in the control group in the distribution study and in all groups in the disease study. Binding in the striatum and insular cortex in ex- and non-smokers is shown in Table 2. Exposure to neuroleptic medication was also shown not to affect 5-[$^{125}$I]-A-85380 binding, with no significant differences seen between exposed and non-exposed cases within disease groups (Table 3).

### Autoradiography

*Distribution in normal tissue.* 5-[$^{125}$I]-A-85380 binding in normal human brain tissue was highest in the thalamic nuclei (Figure 1b and c), in particular the lateral geniculate (LG) nucleus and anteroprincipal (APr) thalamic nucleus ($1.50 \pm 0.31$ and $1.49 \pm 0.64$ fmol/mg, respectively) where binding is 3- to 4-fold higher than in average cortical binding. Binding in the cerebellum (Figure 1a) was five-fold higher in the molecular layer ($1.17 \pm 0.20$ fmol/mg) than the white matter ($0.20 \pm 0.06$ fmol/mg), with moderate binding in the granular layer ($0.48 \pm 0.06$ fmol/mg). The highest cortical density was in the entorhinal cortex ($0.82 \pm 0.55$ fmol/mg) (Figure 1f). Moderate binding was also found in the visual area (Brodmann Area (Ba) 17) and secondary visual area (Ba18), in particular middle and lower layers ($0.50 \pm 0.08$ and $0.48 \pm 0.126$ fmol/mg), in the occipital lobe (Figure 1e) and moderate binding was also found in the auditory association area (Ba21), Ba20, and Ba36 in the temporal cortex ($0.44 \pm 0.11$, $0.45 \pm 0.17$, and $0.46 \pm 0.08$ fmol/mg, respectively) (Figure 1f). The striatal binding was moderate with slightly higher binding in the caudate ($0.57 \pm 0.17$ fmol/mg) than putamen ($0.56 \pm 0.19$ fmol/mg), whereas binding in the globus pallidus (GP) was largely absent (Figure 1b). Also highlighted with moderate binding were white matter tracts in the occipital (Figure 1e), frontal (Figure 1c), and temporal lobes (Figure 1f) where binding was generally 3- to 5-fold greater than white matter ($0.26 \pm 0.06$ and $0.48 \pm 0.13$ fmol/mg). No measurable non-specific binding of 5-[$^{125}$I]-A-85380 was apparent in any areas studied when binding was displaced by 1 μM epibatidine. An example of non-specific binding in a section of striatum, anterior thalamus, and adjacent tissue is illustrated in Figure 1d.

**Table 2** Average 5-[$^{125}$I]-A-85380 Binding Densities (fmol/mg of Tissue) in Control Ex- and Non-smokers

| Area | Non-smokers (*n*) | Ex-smokers (*n*) |
|---|---|---|
| Caudate | $0.65 \pm 0.16$ (6) | $0.41 \pm 0.00$ (3) |
| Putamen | $0.62 \pm 0.21$ (6) | $0.39 \pm 0.07$ (3) |
| Insular cortex | $0.30 \pm 0.14$ (5) | $0.24 \pm 0.02$ (3) |

There are no significant differences between the groups (one-way ANOVA). *n* = number of cases.

**Table 3** Average 5-[$^{125}$I]-A-85380 Binding Densities (fmol/mg of Tissue) in Known Neuroleptic Exposed and Non-exposed AD and DLB Cases

| | | Neuroleptic drug history (*n*) | |
|---|---|---|---|
| Disease group | Area | Exposed | Nonexposed |
| AD | Caudate | $0.43 \pm 0.06$ (3) | $0.42 \pm 0.11$ (4) |
| | Putamen | $0.46 \pm 0.07$ (3) | $0.46 \pm 0.03$ (3) |
| | Insular cortex | $0.28 \pm 0.08$ (3) | $0.28 \pm 0.08$ (3) |
| DLB | Caudate | $0.41 \pm 0.14$ (9) | $0.35 \pm 0.10$ (12) |
| | Putamen | $0.40 \pm 0.13$ (9) | $0.36 \pm 0.11$ (12) |
| | Insular cortex | $0.41 \pm 0.11$ (9) | $0.34 \pm 0.12$ (13) |

There are no significant differences related to exposure (one-way ANOVA). *n* = number of cases.

*Disease comparisons.* Striatal 5-[$^{125}$I]-A-85380 binding in AD, DLB, and PD tended to be lower than both control and VaD cases (Table 4), reaching significance in the caudate ($F(6,58) = 6.8$, $p < 0.05$, $< 0.001$, and $< 0.001$, respectively) and in the putamen in only the DLB and PD groups ($F(6,60) = 6.94$, $p < 0.001$ and $< 0.001$, respectively). In addition, 5-[$^{125}$I]-A-85380 binding in the putamen was significantly lower in PD than in AD ($F(6,60) = 6.94$, $p < 0.05$). In the entorhinal cortex, significantly lower 5-[$^{125}$I]-A-85380 binding was shown in AD, DLB, and PD compared to controls ($F(5,11) = 2.03$, $p < 0.05$). The DLB group also showed significantly lower 5-[$^{125}$I]-A-85380 binding than controls in the ventrolateral posterior thalamic (VLP) nucleus ($F(6,18) = 1.16$, $p < 0.05$) and the SN ($F(6,38) = 4.06$, $p < 0.01$). In the SN, 5-[$^{125}$I]-A-85380 binding was also significantly reduced in DLB compared to AD and VaD cases ($F(6,38) = 4.06$, $p < 0.05$ and $< 0.001$, respectively). In the SN, significantly lower 5-[$^{125}$I]-A-85380 binding was observed in PD compared with control, AD. and VaD cases ($F(6,38) = 4.06$, $p < 0.0$, $< 0.01$, and $p < 0.001$, respectively). The PD group also was shown to have significantly reduced 5-[$^{125}$I]-A-85380 binding than controls in the subthalamic (STH) nucleus ($F(3,35) = 1.58$, $p < 0.05$), the reticular nucleus ($F(6,62) = 1.02$, $p < 0.05$), and the APr thalamic nucleus ($F(6,29) = 1.67$, $p < 0.05$). 5-[$^{125}$I]-A-85380 binding in the STH nucleus and insular cortex in PD was shown to be significantly lower than in DLB ($F(3,35) = 1.58$, $p < 0.05$ and $F(6,60) = 2.16$, $p < 0.01$, respectively). No significant difference in 5-[$^{125}$I]-A-85380 binding was seen between the AD and mild AD groups in all areas studied.

## DISCUSSION

### Effect of Drug and Smoking Histories on 5-[$^{125}$I]-A-85380 Binding

The density of 5-[$^{125}$I]-A-85380 binding in ex-smokers was similar to that in non-smokers in all areas and groups investigated, in agreement with the reported similar

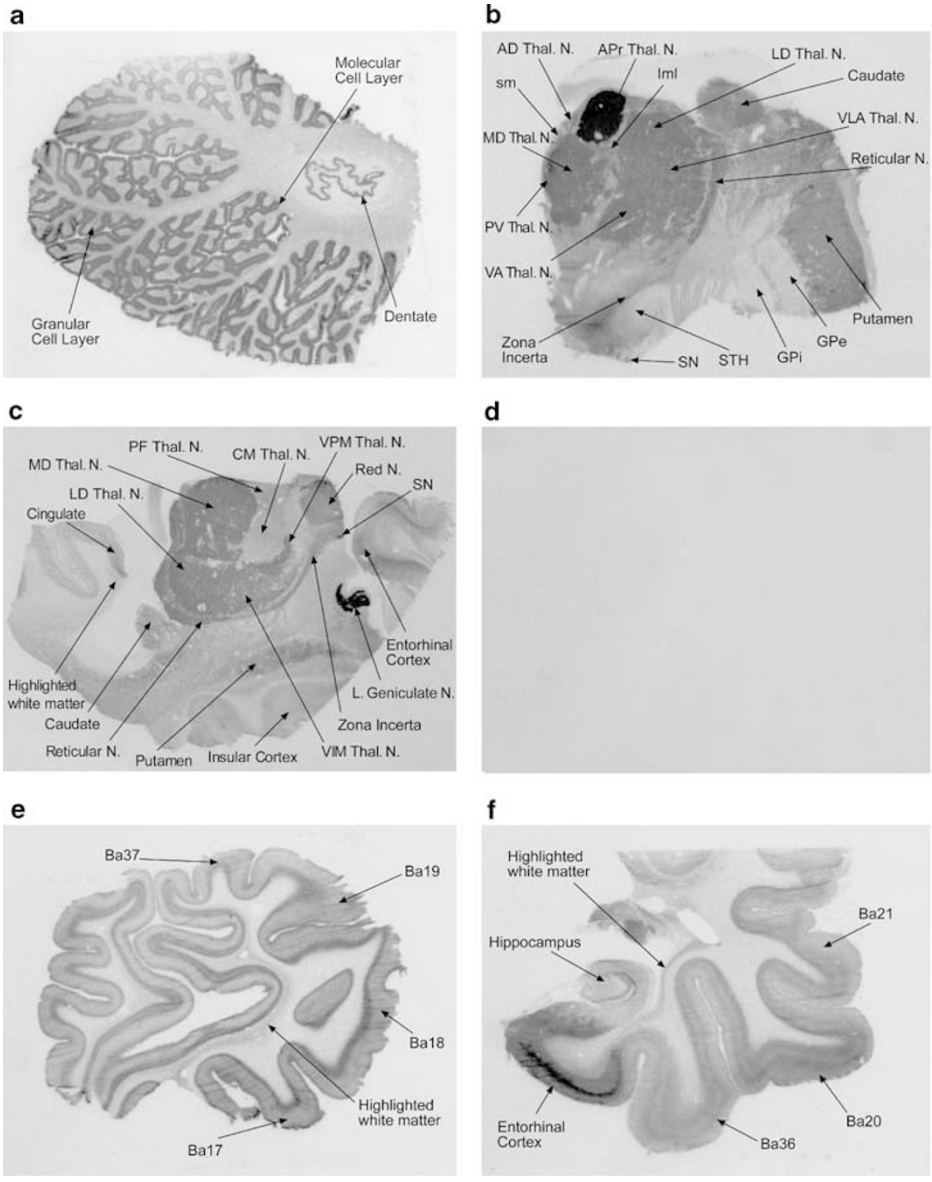


**Figure 1** Autoradiographs of 5-[$^{125}$I]-A-85380 binding in human tissue sections: (a) cerebellum, (b) striatal section showing caudate, putamen, anterior thalamus, subthalamic nucleus, and the edge of the substantia nigra, (c) thalamic section showing lateral geniculate and cingulate, entorhinal and insular cortices, (d) (c) when displaced with 1 μM epibatidine, (e) occipital lobe, (f) temporal lobe. (AD = anterodorsal, APr = anteroprincipal, CM = centromedian, GPe = external globus pallidus, GPi = internal globus pallidus, Iml = internal medullary lamina, L. = lateral, LD = lateral dorsal, MD = medial dorsal, N. = nucleus, PF = parafascicular, PV = paraventricular, sm = stria medullaris, SN = substantia nigra, STH = subthalamic nucleus, Thal. = thalamic, VA = ventroanterior, VIM = ventrointermedius, VLA = Ventrolateral anterior, VPM = Ventroposterior medial).

[$^3$H]nicotine binding in human brain in non- and ex-smokers (Court *et al*, 1998). In addition, there were no significant differences in 5-[$^{125}$I]-A-85380 binding between patients exposed and not exposed to neuroleptic medication in all groups and areas studied. Previous post-mortem studies have shown neuroleptic medication to be associated with accentuation of [$^3$H]nicotine binding deficits in the caudate nucleus (Court *et al*, 2000b). Results from this study suggest that neuroleptic medication may affect nAChRs other than the $\alpha4\beta2$ subtype.

## Distribution in Normal Tissue

*In vitro* visualization of 5-[$^{125}$I]-A-85380 receptor distribution has previously been carried out in rats (Mukhin *et al*, 2000) and mice (Musachio *et al*, 1998; Horti *et al*, 1999). In the present study, the binding distribution of 5-[$^{125}$I]-A-85380 in human post-mortem tissue was found to be consistent with [$^3$H]cytisine (Pabreza *et al*, 1991), [$^3$H]epibatidine (Marutle *et al*, 1998), and [$^3$H]nicotine (Spurden *et al*, 1997; Court *et al*, 2000a) binding, with highest levels appearing in the thalamus, in particular the LG nucleus and APr nucleus, and the entorhinal cortex.

5-[$^{125}$I]-A-85380 binding in the caudate and putamen was of intermediate density, similar to reported [$^3$H]epibatidine (Marutle *et al*, 1998) and [$^3$H]nicotine (Court *et al*, 1998, 2000a) binding in human brain tissue. A recent study by Kulak *et al* (2002) has shown 5-[$^{125}$I]-A-85380 binding to α-conotoxin MII-sensitive nAChRs in the rodent and monkey striatum. These α-conotoxin MII-sensitive sites contain the

**Table 4** Average Binding Densities (fmol/mg of Tissue) of 5-[$^{125}$I]-A-85380 in Areas in the Striatum, Anterior Thalamus, and Temporal Cortex in Control, DLB, AD, Mild AD, VaD, and PD Cases

| | Av binding densities | | | | | |
|---|---|---|---|---|---|---|
| **Area** | **Control (*n*)** | **DLB (*n*)** | **AD (*n*)** | **Mild AD (*n*)** | **VaD (*n*)** | **PD (*n*)** |
| Caudate | 0.57 ± 0.17 (12) | 0.37 ± 0.12 (23)$^{***\#\#\#}$ | 0.43 ± 0.08 (8)$^{*\#}$ | 0.59 ± 0.23 (4) | 0.61 ± 0.12 (6) | 0.30 ± 0.11 (9)$^{***\#\#\#}$ |
| Putamen | 0.56 ± 0.19 (12) | 0.37 ± 0.11 (23)$^{***\#\#\#}$ | 0.47 ± 0.05 (7) | 0.48 ± 0.12 (4) | 0.55 ± 0.09 (10) | 0.30 ± 0.08 (9)$^{***\dagger\#\#\#}$ |
| External GP | 0.16 ± 0.05 (11) | 0.16 ± 0.05 (21) | 0.15 ± 0.03 (6) | 0.17 (2) | 0.16 ± 0.03 (10) | 0.10 ± 0.04 (7)$^{*\Phi\#}$ |
| Internal GP | 0.11 ± 0.03 (8) | 0.13 ± 0.10 (16) | 0.11 ± 0.03 (6) | 0.10 (2) | 0.11 ± 0.03 (7) | 0.06 ± 0.02 (6)$^{\Phi}$ |
| Insular cortex | 0.30 ± 0.11 (10) | 0.36 ± 0.11 (24) | 0.30 ± 0.08 (7) | 0.30 ± 0.10 (4) | 0.31 ± 0.05 (10) | 0.25 ± 0.09 (9)$^{\Phi\Phi}$ |
| Cingulate | 0.45 ± 0.03 (3) | 0.57 ± 0.14 (13) | 0.47 ± 0.05 (2) | — | — | — |
| VA thalamic N | 0.73 ± 0.10 (6) | 0.69 ± 0.16 (13) | 0.70 ± 0.15 (5) | — | 0.87 ± 0.23 (6) | 0.59 ± 0.27 (4)$^{\#}$ |
| APr thalamic N. | 1.49 ± 0.63 (7) | 1.23 ± 0.44 (13) | 1.04 ± 0.30 (4) | 1.10 (2) | 1.33 ± 0.36 (3) | 0.78 ± 0.21 (5)* |
| VLP thalamic N. | 0.80 ± 0.28 (7) | 0.60 ± 0.11 (9)* | 0.66 ± 0.07 (3) | — | — | 0.54 ± 0.10 (3) |
| STH N. | 0.26 ± 0.10 (11) | 0.26 ± 0.09 (13) | 0.21 ± 0.06 (4) | 0.20 (2) | 0.22 ± 0.08 (5) | 0.16 ± 0.05 (5)$^{*\Phi}$ |
| SN | 0.29 ± 0.08 (10) | 0.19 ± 0.05 (14)$^{**\dagger\#\#\#}$ | 0.30 ± 0.15 (5) | 0.25 (2) | 0.33 ± 0.07 (6) | 0.16 ± 0.05 (6)$^{**\dagger\dagger\#\#\#}$ |
| Reticular N. | 0.67 ± 0.20 (12) | 0.62 ± 0.15 (24) | 0.59 ± 0.09 (8) | 0.65 ± 0.19 (4) | 0.68 ± 0.17 (8) | 0.53 ± 0.12 (10)* |
| Entorhinal cortex | 0.82 (2) | 0.39 ± 0.15 (6)* | 0.40 ± 0.05 (3)* | — | — | 0.32 (2)* |

The known smoker has been eliminated from these data and ex-smokers are included. Apr = anteroprincipal, GP = globus pallidus, SN = substantia nigra, STH = subthalamic, VA = ventral anterior, VLP = ventrolateral posterior, *n* = number of cases.
*Significantly lower than the control group, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (ANOVA: single factor).
$^{\dagger}$Significantly lower than the AD group, $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ (ANOVA: single factor).
$^{\Phi}$Significantly lower than the DLB group, $^{\Phi}p<0.05$, $^{\Phi\Phi}p<0.01$ (ANOVA: single factor).
$^{\#}$Significantly lower than the VaD group, $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ (ANOVA: single factor).

α6 and β2 subunits (Grady *et al*, 2001; Champtiaux *et al*, 2002) and therefore striatal binding evident in the present study is likely to reflect both α4β2 and α6β2 subtype distributions. [$^{125}$I]-α-conotoxin MII binding in mouse brain has also been shown to be high in the superficial layers of the superior colliculus, nigrostriatal pathways, optic tract, olivary pretectal, mediolateral and dorsolateral geniculate nuclei (Whiteaker *et al*, 2000) and therefore 5-[$^{125}$I]-A-85380 in these areas could also reflect both α4β2 and α6β2 subtype distributions. 5-[$^{125}$I]-A-85380 binding in the GP is largely absent, which is consistent with low [$^{3}$H]nicotine binding in human brain tissue (Court *et al*, 2000a).

In the cerebellum, 5-[$^{125}$I]-A-85380 binding appears more concentrated in the molecular layer than the granular layer, confirming previously reported [$^{3}$H]cytisine (Aubert *et al*, 1992a) and [$^{3}$H]epibatidine distribution (Court *et al*, 2000a); however, [$^{3}$H]nicotine binding shows higher binding in the granular than the molecular layer (Court and Perry, 1995a). Moderate 5-[$^{125}$I]-A-85380 binding was also present in the dentate nucleus. Immunohistochemical localization of nicotinic receptors in the cerebellum has shown a number of receptor subunits, including both α4 and α6, to be present in molecular and granule cell layer (Graham *et al*, 2002) and therefore 5-[$^{125}$I]-A-85380 binding in the cerebellum may not be solely due to α4 but also to α6 subunit containing receptors. Cerebellar dysfunction has been implicated in attentional deficits. Nicotinic receptor agonists enhance attentional function and in autism α4 subunit abnormalities have been highlighted in the cerebellum (Lee *et al*, 2002) and therefore 5-I-A-85380 imaging may be useful in identifying abnormalities in this area *in vivo*.

In the occipital cortex, 5-[$^{125}$I]-A-85380 binding appears to be concentrated in the visual cortex (Ba17) and the secondary visual area (Ba18), with highest levels in lower cortical layers, consistent with the specific action of acetylcholine in vision (Nobili and Sannita, 1997). Acetylcholine is thought to serve both sensory and cognitive processes involved in vision, and 5-[$^{125}$I]-A-85380 binding in the LG nucleus may reflect the 'gating' of sensory information to the visual cortex.

Binding of 5-[$^{125}$I]-A-85380 in white matter in the temporal, occipital, and cingulate cortices appears to course with cholinergic pathway previously observed using acetylcholinesterase histochemistry (Selden *et al*, 1998). Binding of 5-[$^{125}$I]-A-85380 shown in the temporal cortex follows the course of the capsular division of the lateral cholinergic pathway. Fibers of this pathway run adjacent to the putamen in the medial aspect of the external capsule into the nucleus basalis of Meynert which then project down into the temporal cortex (Selden *et al*, 1998). Binding of 5-[$^{125}$I]-A-85380 to fibers in the external capsule was not clearly seen due to moderate binding seen in the putamen and claustrum; however, binding to fibers running from the nucleus basalis of Meynert into the temporal cortex was clearly highlighted. The binding of 5-[$^{125}$I]-A-85380 in the occipital lobe white matter correlates with lateral and medial cholinergic pathways merging posteriorly in the occipital lobe (Selden *et al*, 1998). 5-[$^{125}$I]-A-85380 binding at the tip of the cingulate gyrus correlates with the medial cholinergic pathway supplying the cingulate gyrus (Selden *et al*, 1998). Streaks of intense [$^{3}$H]nicotine white matter binding previously seen in foetal cerebellum and temporal lobe may also reflect the presence of nAChRs along

cholinergic axons (Court *et al*, 1995b, 1997), probably visible in fetal tissue because quenching of [$^3$H]nicotine is reduced due to lower myelination. It is reasonable to conclude that neuronal $\alpha4\beta2$ (or possibly $\alpha6\beta2$) receptors are located along cholinergic nerve fiber tracts possibly indicative of preassembled receptors being transported from the cell body along axons to terminals. A cholinergic fiber loss in cortical areas has previously been reported in AD brains compared to controls (Geula and Mesulam, 1996) and therefore 5-I-A-85380 may be useful in highlighting these deficits *in vivo* in humans. Further investigation into cortical areas in disease post-mortem tissue may show 5-I-A-85380 to be useful diagnostically, highlighting deficits in cholinergic pathways.

## Disease Comparisons

*Alzheimer's disease.* Reports on the density of nAChRs in the striatum in AD are inconsistent with studies reporting both loss and preservation of agonist binding in the caudate and putamen (Shimohama *et al*, 1986; Perry *et al*, 1989; Rinne *et al*, 1991; Aubert *et al*, 1992a). In this study, posterior striatal levels were investigated and deficits in 5-[$^{125}$I]-A-85380 binding in the caudate differentiated the AD group from controls. Deficits in striatal nAChRs do not appear, at least in some patients, to be associated with nigrostriatal dopaminergic deficits (Piggott *et al*, 1999) and are therefore more likely to be related to reduced striatal afferents from other sources, such as cortical areas subject to atrophy in AD and possibly thalamic areas. It may be that these striatal nAChRs deficits are responsible for disorders of movement observed in late stages of AD (Court *et al*, 2000a). Significantly reduced 5-[$^{125}$I]-A-85380 binding in AD compared to controls was also found in the entorhinal cortex, consistent with reports of deficits in [$^3$H]nicotine binding (Court *et al*, 2001) and [$^3$H]epibatidine binding and $\alpha4$ subunit protein expression in the neocortex in AD (Martin-Ruiz *et al*, 1999; Guan *et al*, 2000). A trend of lower 5-[$^{125}$I]-A-85380 in thalamic nuclei was seen in AD; however, this did not reach statistical significance confirming earlier reports of no major deficits in thalamic areas in AD (Xuereb *et al*, 1990; Court *et al*, 2001).

*Mild alzheimer's disease.* There were no significant differences in 5-[$^{125}$I]-A-85380 binding in mild AD compared to controls and also AD patients. This lack of change in binding in mild AD cases suggests that 5-[$^{125}$I]-A-85380 may not be useful for early differential diagnosis. It must be noted, however, that only four mild AD cases were studied and investigation into areas where nAChRs are reported to be most markedly reduced in AD, such as temporal cortex, hippocampus, and entorhinal cortex, may still show 5-[$^{125}$I]-A-85380 to be useful in this respect. *In vivo* 5-[$^{125}$I]-A-85380 SPECT imaging, however, could potentially be useful in the assessment of effects of anticholinesterase therapy.

*Lewy body dementia.* Reduced 5-[$^{125}$I]-A-85380 binding in both the caudate and putamen differentiated DLB from controls corroborating evidence of similar reductions in [$^3$H]nicotine binding (Court *et al*, 2000b). In DLB, deficits in striatal M1 muscarinic acetylcholine receptors (mAChRs), measured using [$^3$H]pirenzepine binding, have also been demonstrated (Piggott *et al*, 2003), highlighting a postsynaptic deficit, whereas 5-[$^{125}$I]-A-85380 binding is likely to indicate a presynaptic deficit. In the SN, a region composed of dopaminergic nuclei projecting to the striatum, reduced 5-[$^{125}$I]-A-85380 binding differentiated DLB from controls and also AD cases. The relative severity of reduced 5-[$^{125}$I]-A-85380 binding seen in both the striatum and substantia nigra in DLB, comparable to that in PD, could potentially be a marker of early degeneration, possibly highlighting deficits in nigrostriatal inputs, as SN dopaminergic neuron loss is greater in PD than in DLB (Piggott *et al*, 1999), or deficits in other cortical inputs.

A reduction in 5-[$^{125}$I]-A-85380 binding in the entorhinal cortex in DLB cases compared to controls confirms evidence of reductions in [$^3$H]nicotine and [$^3$H]epibatidine binding in this brain area in DLB (Perry *et al*, 1995; Martin-Ruiz *et al*, 2000). The relative severity of the reduction of [$^3$H]epibatidine binding in the temporal cortex has been reported to be related to disturbances in consciousness (DOC) seen in some DLB patients, where patients with DOC show higher binding in the temporal cortex, including the entorhinal cortex, than patients showing no DOC (Ballard *et al*, 2002). Imaging with 5-[$^{123}$I]-A-85380 could potentially highlight these differences *in vivo*, and clinical correlative studies in DLB are in progress.

*Parkinson's disease.* Deficits in 5-[$^{125}$I]-A-85380 binding in both the caudate and the putamen differentiated PD from controls, as in DLB; however, lower 5-[$^{125}$I]-A-85380 binding in the putamen also differentiated PD from AD cases. This result in PD is not unexpected due to the presence of presynaptic nAChRs on striatal dopaminergic terminals (Wonnacott, 1997). The loss of striatal 5-[$^{125}$I]-A-85380 binding of 46–50% closely parallels previously reported [$^3$H]nicotine reductions (Court *et al*, 2000b) and also the loss of nigrostriatal dopaminergic markers (Piggott *et al*, 1999). A study with 5-[$^{125}$I]-A-85380 and [$^{125}$I]epibatidine in control human and PD brains showed a similar decrease of 40–50% in binding of both radioligands in the striatum (Quik *et al*, 2002).

In the SN, reduced 5-[$^{125}$I]-A-85380 binding also differentiated PD from controls and AD cases, highlighting the loss of dopaminergic nuclei projecting to the striatum. This finding is in agreement with evidence of reduced [$^3$H] nicotine binding in the SN pars compacta and ventral tegmental area (Perry *et al*, 1995).

Reduced 5-[$^{125}$I]-A-85380 binding in the entorhinal cortex also differentiates PD from controls, supporting a number of studies demonstrating a deficit in nAChRs with high-affinity agonists and ChAT activity in the neocortex in PD (Whitehouse *et al*, 1988; Rinne *et al*, 1991; Aubert *et al*, 1992b; Perry *et al*, 1993; Lange *et al*, 1993).

Thalamic 5-[$^{125}$I]-A-85380 binding in the PD group tended to be lower than controls, with significance reached in the APr thalamic nucleus, the reticular nucleus, and also STH nucleus. Thalamic 5-[$^{125}$I]-A-85380 binding in PD also tended to be lower than in DLB, with significance reached in the STH nucleus. Dopamine deficiency in PD causes the disinhibition and overactivity of the STH nucleus and its projections to the internal GP, which in turn overinhibits

thalamocortical projections which is thought to be responsible for the appearance of symptoms such as akinesia, rigidity, and perhaps tremor (Henderson and Dunnett, 1998). A deficit in 5-[$^{125}$I]-A-85380 binding in the STH nucleus in PD emphasizes the potential involvement of acetylcholine in the expression of these symptoms. Reduced 5-[$^{125}$I]-A-85380 binding in the insular cortex also differentiated the PD group from the DLB group.

*Vascular dementia:* Although 5-[$^{125}$I]-A-85380 binding in thalamic areas tended to be lower in a number of thalamic nuclei, no significant reductions were found for VaD in comparison with controls in any brain area investigated, supporting evidence of no reductions in either [$^{3}$H]epibatidine binding or $\alpha$4 nAChR subunit expression in the temporal cortex in VaD (Martin-Ruiz *et al*, 2000). Reduced 5-[$^{125}$I]-A-85380 binding in striatal areas in AD, DLB, and PD and in the substantia nigra in DLB and PD differentiates these groups from VaD as well as controls. This could be valuable in differentiating VaD from primary degenerative dementia. It must be noted, however, that these differences were not seen between mild AD and VaD.

In summary, 5-[$^{125}$I]-A-85380 binding reflects the previously reported distribution of $\alpha4\beta2$ receptors in human brain and also differences of nAChRs seen with high-affinity agonists in age-related degenerative diseases. Significant differences were not only observed between dementia cases and controls but also between cases with different causes of dementia. Therefore, 5-[$^{125}$I]-A-85380 is potentially a useful *in vivo* tool to aid specific diagnosis that is important in terms of treatment and management strategies.

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