ELSEVIER

Pharmacology, Biochemistry and Behavior 70 (2001) 571–579

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Visual hallucinations are associated with lower αbungarotoxin binding in dementia with Lewy bodies

J.A. Court[a,*], C.G. Ballard[b], M.A. Piggott[a], M. Johnson[a], J.T. O'Brien[b], C. Holmes[c], N. Cairns[d], P. Lantos[d], R.H. Perry[e], E. Jaros[e], E.K. Perry[a]

[a]*Joint MRC–University of Newcastle Centre Development in Clinical Brain Ageing, MRC Building, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, UK*

[b]*Wolfson Research Centre, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, UK*

[c]*Thornhill Research Unit, Faculty of Medicine and Biological Science, Moor Green Hospital, University of Southampton, Southampton SO30 3JB, UK*

[d]*Department of Pathology, Institute of Psychiatry, Maudsley Hospital, London SE5 8AZ, UK*

[e]*Department of Neuropathology, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, UK*

Received 4 March 2001; received in revised form 17 June 2001; accepted 19 June 2001

Abstract

Patients with dementia with Lewy bodies (DLB) commonly experience psychotic symptoms, most notably visual hallucinations. Previously, it has been shown that visual hallucinations in DLB are associated with reduced cortical choline acetyltransferase activity, a marker of cholinergic innervation, but not with predominantly postsynaptic muscarinic M1 receptor binding. In the present investigation, nicotinic acetylcholine receptor (nAChR) levels in the temporal cortex (Brodmann's areas [BA] 20 and 36) were measured in a group of 24 prospectively assessed DLB patients; comparisons were made between groups with or without visual and auditory hallucinations and delusional misidentification. Visual hallucinations and delusional misidentification were associated with lower [^{125}I]αbungarotoxin binding in areas 36 and 20 ($P<.05$), but not with changes in [^{3}H]epibatidine binding. There were no significant associations with auditory hallucinations. [^{3}H]epibatidine, but not [^{125}I]αbungarotoxin, binding for all DLB cases was reduced compared to controls ($P<.001$). Loss of cortical α7 nicotinic receptors may contribute to hallucinations and delusional misidentification in DLB, with implications for treatment and understanding the mechanisms of psychotic symptoms in dementia. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Nicotinic acetylcholine receptors; α7 subunits; Dementia with Lewy bodies; Visual hallucinations; Delusional misidentification; Temporal cortex

1. Introduction

Dementia is a disabling and distressing disorder that affects 5% of the population over the age 65 and 20% of those over 80 (Cummings and Benson, 1992) with substantial emotional (Pearlin et al., 1990) and financial cost (Gray and Fenn, 1993). The two most common forms of degenerative dementia are Alzheimer's disease (AD), which constitutes approximately 60% of cases, and dementia with Lewy bodies (DLB), accounting for a further 10–15% of sufferers (McKeith et al., 1996). Psychotic symptoms such as hallucinations and delusional misidentification are frequent in patients with dementia. They are distressing for patients (Gilley et al., 1991) and their carers (Rabins et al., 1982) in whom they are associated with additional psychiatric morbidity (Deimling and Bass, 1986). Furthermore, these symptoms often occur in conjunction with behavioural disturbances such as aggression or agitation (Rockwell et al., 1994). Dementia patients with psychosis, particularly those experiencing visual hallucinations, have a two- to threefold accelerated rate of cognitive decline (Drevets and Rubin, 1989; Rosen and Zubenko, 1991; Förstl et al., 1993; Chui et al.,1994), and a number of studies have reported an association between psychotic symptoms and admission to residential or nursing home care (Steele et al., 1990; Haupt et al., 1996).

In DLB, psychotic symptoms, including visual hallucinations (>80%), delusional misidentification (>30%) and auditory hallucinations (>30%), are more frequent, distress-

* Corresponding author. Tel.: +44-191-273-5251; fax: +44-191-272-5291.

E-mail address: j.a.court@ncl.ac.uk (J.A. Court).

PII: S0091-3057(01)00644-X

ing and persistent than in other types of dementia such as AD and vascular dementia (Ballard et al., 1995, 1999). Whilst visual hallucinations and delusional misidentification both appear to be related to abnormalities of visual processing (Förstl et al., 1991), in DLB patients when auditory hallucinations are present they usually co-occur with hallucinations in the visual modality (Ballard et al., 1997b); the close relationship of these three phenomena suggests the possibility of a shared biological basis.

The clinical management of psychosis is particularly problematic in patients with DLB, with extreme sensitivity reactions (characterised by delirium, severe parkinsonism, cognitive decline and often death) occurring in response to typical and atypical neuroleptic drugs in 25–50% of cases (McKeith et al., 1992, 1995; Ballard et al., 1998). Identifying novel, safe and effective therapeutic agents for psychosis in DLB is thus imperative.

Traditionally, disturbances in dopaminergic transmission have been considered to underlie psychotic phenomena, including hallucinations, in people with dementia. However, there is only limited evidence that L-dopa can exacerbate hallucinations in Parkinson's disease (Zoldan et al., 1996) and there is no distinction between hallucinating and nonhallucinating patients with respect to L-dopa treatment (Haeske-Dewick, 1995), and increasing L-dopa is not associated with increased frequency of hallucinations (Goetz et al., 1998). Furthermore, studies directly evaluating the link between psychosis and dopaminergic function in AD have not identified any significant associations with dopaminergic parameters (Zubenko et al., 1991).

There is very little specific information regarding the impact of L-dopa upon visual hallucinations in DLB. However, previous analysis of autopsy brain tissue has identified more extensive reductions of presynaptic cholinergic activity in DLB compared to AD (Perry et al., 1990b) and greater cholinergic deficits in the cortex of hallucinating compared to nonhallucinating DLB patients together with a relative overactivity of the 5-HT system (Perry et al., 1990a), indicating that although other factors such as L-dopa administration could possibly play an adjunctive role, acetylcholine is probably more important than dopamine as a substrate of hallucinations in these patients. Consistent with these observations, hallucinations can be induced by cholinergic antagonists acting on muscarinic receptors (Perry and Perry, 1995), although whether nicotinic receptor antagonists have a similar hallucinogenic effect is unknown. In addition, in preliminary studies visual hallucinations in DLB patients are particularly responsive to anticholinesterase therapy (Fergusson and Howard, 2000; Kaufer et al., 1998; Shea et al., 1998: Levy et al., 1994). Similar results have been reported for tacrine in Parkinson's disease patients with dementia (Hutchinson and Fazzini, 1996) and for metrifonate in AD (Morris et al., 1998).

In view of previous studies indicating a link between cholinergic deficits and visual hallucinations, there is a clear need to examine specific components of the cholinergic system in more detail. A recent study by our group did not identify an association between changes in muscarinic receptor subtypes and hallucinations (Ballard et al., 2000). To date the potential role of nicotinic acetylcholine receptors (nAChRs) in relation to psychotic symptoms in dementia has not been examined. Two major subtypes of nAChRs are present in human brain, those that bind nicotinic agonists (such as nicotine, cytisine and epibatidine) with high affinity and those that bind the antagonist αbungarotoxin (αBGT), and are considered to be associated with α4β2 and α7 receptor subunits, respectively (reviewed, Paterson and Nordberg, 2000). In DLB, loss of nicotinic agonist binding ([^{3}H]epibatidine and [^{3}H]nicotine) has been observed in parietal cortex (Perry et al., 1990b), hippocampal formation (Perry et al., 1995) and, together with αBGT binding, in the mid-frontal gyrus (Reid et al., 2000) compared to aged matched controls. Reduced αBGT binding in DLB has also been observed in the reticular nucleus of the thalamus (Court et al., 1999). Recent genetic and autopsy studies of schizophrenia, another condition with prominent delusions and hallucinations, indicate that the α7 nicotinic receptors and possibly α4β2 subtypes may also have an important role (Freedman et al., 1995, 1997; Leonard et al., 1996; Court et al., 1999, 2000; Guan et al., 1999; Durany et al., 2000). Although the phenomenology of psychotic symptoms is different in the schizophrenia and DLB (e.g., rarity of first rank symptoms and predominance of visual hallucinations in DLB and high frequency of first rank symptoms and auditory hallucinations in schizophrenia), both subtypes of nAChRs are potential candidates as neurochemical substrates of hallucinations in DLB. The predominance of visual hallucinations in DLB, as opposed to auditory in schizophrenia, may reflect the different neural pathways that are affected.

The relationship between nAChRs and both hallucinations and delusional misidentification was evaluated in a prospectively assessed series of patients diagnosed clinically (with subsequent neuropathological confirmation) as having DLB. Nicotinic receptor densities were examined in areas temporal cortex (Brodmann's areas [BA] 20 and 36), adjacent to the hippocampus that have been implicated in encoding complex visual and visual object recognition (Ellerman et al., 1998), by postmortem autoradiography using [^{3}H]epibatidine and [^{125}I]αBGT. Measurements in DLB cases were also compared with age-matched normal controls. We hypothesised that there would be a significant association between loss of nAChRs in the temporal cortex and visual hallucinations.

2. Methods

2.1. Cases

The study was been fully approved by a human subjects ethical committee. Amongst a representative clinical cohort

of 420 patients from hospital dementia case registers in Newcastle (Institute for the Health of the Elderly, $n=340$) and London (Institute of Psychiatry, $n=80$), 24 with a clinical diagnosis of DLB (McKeith et al., 1996) were followed to autopsy, 23 of whom had received detailed prospective evaluation during life. Of the 24 cases, 16 (67%) were female, their mean age at assessment was 78.0 ± 6.0, the average time from first assessment to death was 5.2 ± 1.3 years, the mean Mini-Mental State Examination (MMSE) at first assessment was 11.0 ± 7.5 and the mean MMSE closest to death was 6.6 ± 8.8. There were no differences in severity of cognitive impairment or gender between the DLB subgroups with or without the various psychotic phenomena, although patients with visual hallucinations or delusional misidentification were slightly younger (for visual hallucinations 76 ± 5 vs. 83 ± 6 and delusional misidentification: 76 ± 5 vs. 82 ± 6; cases described in Table 1). None of the DLB patients smoked tobacco within 6 months of death. These DLB cases were compared with 10 controls with no evidence of cognitive impairment, psychiatric or neurological disorder (based on detailed evaluation of clinical cases notes), no significant neuropathology (of neurovascular, Alzheimer, Parkinson and DLB-type) and matched for age at death (80 ± 10 and 80 ± 6, respectively) and postmortem delay (37 ± 26 and 40 ± 23 h, respectively). Four of the controls were confirmed to be nonsmokers. Causes of death were predominantly heart disease and respiratory failure in both controls and DLB cases.

2.2. Neuropathological assessment

The right hemisphere was formalin fixed for at least 3 months for neuropathological assessment. The neuropathological diagnosis of DLB was made according to the international consensus criteria (McKeith et al., 1996). The quantitative assessment of cortical Lewy body density followed the consensus protocol (McKeith et al., 1996), using ubiquitin (Daco), Tau2 (Sigma) and AT8 (Endogen), together with α-synuclein (Novacastra) antibodies. The densities of Lewy bodies and α-synuclein positive neurites were assessed using a semiquantitative scale from 0 to 4 (0 *none*, 1 *few*, 2 *moderate*, 3 *high*, 4 *very high*). The evaluation of concurrent AD pathology was made using quantitative techniques to determine plaque and tangle densities (Perry et al., 1990c) and according to the Mirra et al. (1991) (CERAD) criteria. Braak staging was completed for the Newcastle cases (Braak and Braak, 1991). A

Table 1
A summary of the DLB case details (at the time of last clinical assessment)

Gender	Age	MMSE	Visual hallucinations	Delusional misidentification	Auditory hallucinations	Dopaminergic medication	Anticholinergic medication
F	77	22	Yes	No	No	Yes[a]	Yes[b]
F	78	22	Yes	Yes	Yes	Yes[a]	Yes[c]
F	74	3	Yes	Yes	Yes	No	Yes[c]
F	70	12	Yes	Yes	Yes	Yes[a]	No
F	82	0	Yes	Yes	No	No	Yes[b]
M	75	15	Yes	Yes	No	No	No
F	70	6	Yes	Yes	Yes	Yes[a]	Yes[c]
F	81	12	Yes	Yes	Yes	No	Yes[c]
F	81	14	Yes	No	Yes	Yes[a]	Yes[c]
F	88	8	Yes	Yes	No	No	Yes[c]
F	75	11	Yes	Yes	Yes	Yes[a]	No
M	77	25	No	No	No	No	No
F	75	16	Yes	Yes	No	No	No
M	66	<10*	Yes	Yes	No	Yes[a,d]	Yes[b]
M	77	15	Yes	Yes	Yes	Yes[a]	No
F	72	9	Yes	Yes	Yes	Yes[a]	No
M	77	0	Yes	Yes	Yes	No	No
F	78	1	Yes	Yes	No	No	Yes[c]
F	92	0	No	No	No	No	Yes[c]
F	83	16	No	No	No	No	No
F	88	16	No	No	No	No	No
M	75	16	No	No	No	No	Yes[c]
M	82	4	No	No	No	No	No

Anticholinergic medication: No patients received cholinergic antagonists.
MMSE values were not significantly different between those with and those without visual hallucinations ($z=0.67$, $P=.5$, Mann–Whitney). Postmortem delay was also similar between those with and those without visual hallucinations (35 ± 22 and 41 ± 36, respectively).

[a] L-Dopa.
[b] Tricyclic antidepressant.
[c] Neuroleptic.
[d] Dopamine agonist.
* Patient refused MMSE, estimated score <10.

detailed description of the neuropathological characteristics of the Newcastle patients is shown in Table 2.

2.3. Clinical/psychiatric assessment

Clinical assessments, repeated at annual intervals until death, included: a standardised psychiatric history (History and Aetiology schedule-HAS, Dewey et al., 1992); assessment of cognitive function using the Cambridge Assessment of Mental Disorders in the Elderly, Section B (Roth et al., 1986); a standardised physical examination incorporating the modified Unified Parkinson's Disease Rating Scale (Ballard et al., 1997a); assessment of psychosis using the Columbia University Scale for Psychopathology in Alzheimer's disease (Devanand et al., 1992). The definitions developed by Burns et al. (1990) were used to determine the presence and classification of visual hallucinations, auditory hallucinations and delusional misidentification. Hallucinations were defined as a percept in the absence of a stimulus (further categorised according to modality). Delusional misidentification included Capgras phenomena (the delusional belief that a familiar person, or object, has been replaced by an "impostor") as well as delusional misidentification of television images, mirror images and photographs. It was stipulated that all psychotic phenomena must have been directly reported to the assessor or reported to the informant by the patient, and not inferred from behaviour. For the purposes of the current correlative study only persistent psychotic symptoms, lasting longer than 6 months and occurring in the year prior to death, were considered. None of the DLB cases had schizophrenia prior to dementia.

2.4. Neurochemical analyses

At autopsy 1-cm coronal sections of the left hemisphere were snap-frozen in liquid Arcton cooled in liquid nitrogen and then stored at $-70\,^{\circ}C$ for 1–6 years (mean 4.5 ± 1.1 years for normal elderly controls and 3.8 ± 1.6 years for DLB cases). Cryostat sections (20 μm) from the temporal cortex, BA 20 and BA 36 were air-dried on Vectabond- (Vector Laboratories) treated glass slides. For anatomical identification contiguous sections were stained for Nissl (Cresyl fast violet).

Tissue sections were analysed for nicotinic receptors using [^{125}I]αBGT binding, which reflects the α7 subunits (Clarke, 1992), and [^{3}H]epibatidine, which binds with high affinity to α4β2 receptors and possibly other nAChRs containing additional subunits, e.g., α3 (Zoli et al., 1998). For [^{3}H]epibatidine binding, tissue sections were preincubated in 50 mM Tris–Cl buffer pH 7.4 containing 8 mM $CaCl_2$ at room temperature for 20 min to remove endogenous ligands. Sections were then incubated in the presence of 1 nM [^{3}H]epibatidine in the same buffer for 3 h at room temperature. Nonspecific binding was assessed in the presence of 1 μM cytisine. After incubation, tissue sections were washed for a total of 30 s in three changes of ice-cold buffer, dipped in water, and dried rapidly under a stream of air at room temperature. For [^{125}I]αBGT binding, tissue sections were preincubated in 50 mM Tris–Cl buffer pH 7.4 containing 1 mg/ml bovine serum albumin. Sections were then incubated in the presence of 1.2 nM [^{125}I]αBGT for 2 h at room temperature. Nonspecific binding was assessed in the presence of 2.5 mM nicotine. After incubation, sections were washed for a total of 30 min in six changes of ice-cold buffer, dipped in water, and rapidly dried as above. Sections were opposed to ^{3}H-Hyperfilm for 5 days, and 3 months for [^{125}I]αBGT and [^{3}H]epibatidine binding, respectively, and images quantified using Lynx Densitometry Software (Applied Imaging).

2.5. Statistical analyses

Comparisons between control and DLB groups were evaluated (two-tailed student *t* test) for each neurochemical assay. Given the clinical overlap between visual hallucinations, auditory hallucinations and delusional misidentification, statistical evaluations were undertaken to evaluate the relationship between parameters/areas of interest and recep-

Table 2
Summary of Newcastle DLB cases: neuropathological features

Braak stage	CERAD probable/ definite AD	DLB type (ubiquitin)	DLB type (α-synuclein)	Cortical Lewy body density in temporal cortex units	α-Synuclein positive neurites in temporal cortex units
1	Y	Neocortical	Neocortical	3	3
2	N	Limbic	Neocortical	1	1
3	Y	Neocortical	Neocortical	3	3
3	N	Neocortical	Neocortical	3	4
2	Y	Neocortical	Neocortical	2	2
2	Y	Limbic	Neocortical	1	2
2	Y	Neocortical	Neocortical	3	3
2	Y	Limbic	Neocortical	2	1
2	Y	Neocortical	Neocortical	2	3
3	N	Neocortical	Neocortical	3	4
2	N	Neocortical	Neocortical	1	1
1	Y	Neocortical	Neocortical	2	3
2	N	Neocortical	Neocortical	3	2
4	Y	Neocortical	Neocortical	4	4

tor binding using linear regression analyses. Age (as this tended to be different between groups with and without hallucinations), drug medication usage (L-dopa and neuroleptic medication) and MMSE (as this was variable within patient groups) were also entered into the regression analysis as potentially important confounders. Analysis was carried out using SPSS computerised statistics package, release 6.0.

3. Results

3.1. Comparison of nAChR binding between DLB and control cases

There were no significant differences between the DLB cases and the control group in binding of [^{125}I]αBGT, but [^{3}H]epibatidine binding was significantly reduced in BA 20 and 36 in the DLB compared with the control cases ($P<.001$, Table 3). Tobacco smoking does not appear to impact upon αBGT binding (Court et al., 1998), but can potentially influence nicotine agonist binding. Since none of the DLB patients smoked within 6 months of death, and for 6 out of the 10 control cases there was no smoking history, it is possible that this apparent deficit of cortical [^{3}H]epibatidine binding may reflect a high number of tobacco users in the control group. However, this is not likely since the values of [^{3}H]epibatidine binding in the control group as a whole (Table 3) were similar to that for the four controls that were confirmed nonsmokers (3.4 ± 1.9 and 4.5 ± 2.1 in BA 20 and 36, respectively), indicating that the majority of the control cases were likely to be nonsmokers.

3.2. Hallucinations and delusional misidentification in DLB

Detailed prospective clinical evaluations were available for 23 of the DLB patients (summarised in Table 1). Between the subgroups with or without delusional misidentification, or auditory hallucinations, there were no substantial differences in the numbers exposed to drugs with anticholinergic drugs (8/17 and 4/6 with and without delusional misidentification, 5/10 and 5/13 with and without auditory hallucinations, Table 1). There was a slight excess of people taking drugs with anticholinergic properties in the group with visual hallucinations (10/17 and 2/6 with and without visual hallucinations, Table 1). All the patients taking L-dopa (9/9) experienced visual hallucinations, compared to 57% (8/14), who were not. It was also evident that a higher proportion of patients taking L-dopa experienced auditory hallucinations (7/9 vs. 0/2) and delusional misidentification (7/9 vs. 0/2). There is clearly overlap amongst the different psychotic phenomena. All 15 patients with delusional misidentification also experienced visual hallucinations, although 2 of the patients with visual hallucinations did not experience delusional misidentification. Similarly, all of the patients with auditory hallucinations also had visual hallucinations, although 7 of the patients with visual hallucinations did not experience hallucinations in the auditory modality.

Table 3
Nicotinic receptor binding in the temporal cortex: comparison of DLB cases and controls

	Controls	DLB	Statistical evaluation
[^{125}I]αBungarotoxin binding			
BA 20	2.0 ± 0.54 (9)	2.4 ± 0.90 (24) [↑20%]	$t=1.02$, $P=.32$
BA 36	1.9 ± 0.76 (9)	2.2 ± 0.81 (24) [↑16%]	$t=0.71$, $P=.48$
[^{3}H]Epibatidine binding			
BA 20	4.1 ± 1.43 (10)	2.5 ± 0.84 (23) [↓39%]	$t=-3.95$ $P<.001$
BA 36	4.0 ± 1.65 (10)	1.9 ± 0.98 (23) [↓52%]	$t=-4.57$ $P<.001$

Values are means ± S.D. in femtomoles per milligram tissue; the numbers in parenthesis are the number of determinations.
Values in square brackets indicate the mean percent difference in DLB cases compared to controls.

3.3. Association of hallucinations and delusional misidentification with the levels of nAChR binding

Patients with visual hallucinations had 31% lower [^{125}I]αBGT binding in BA 20 and 36 of the temporal cortex than those without ($P<.05$), but there were no significant differences in [^{3}H] epibatidine binding (Table 4). Differences in [^{125}I]αBGT binding between hallucinating and nonhallucinating DLB patients were also apparent amongst patients who did not receive L-dopa therapy (21% lower in BA 36 and 19% lower in BA 20; [^{125}I]αBGT binding in BA 36 — visual hallucinations: 2.3 ± 0.8 and no visual hallucinations: 2.9 ± 1.0 fmol/mg tissue equivalent; BA 20 — visual hallucinations: 2.6 ± 0.9 and no visual hallucinations: 3.2 ± 0.9 fmol/mg tissue equivalent). Differences in [^{125}I]αBGT binding amongst the subgroups were unlikely to be the result of differences in postmortem delay as these were similar for those with and without visual hallucinations (Table 1).

The subgroup of DLB patients with auditory hallucinations also tended to have lower [^{125}I]αBGT binding in BA 20 (26%) and 36 (21%) (Table 4), but these apparent differences were not statistically significant. There were also no significant differences in [^{3}H]epibatidine binding between the subgroups with and without visual hallucinations.

DLB patients with delusional misidentification had lower [^{125}I]αBGT binding in BA 20 (30%) and 36 (30%) compared to those without ($P<.05$, Table 4). These differences between DLB patients with and without delusional misidentification were similar amongst patients who did not receive L-dopa therapy, with a reduction of 29% in BA 36 and 27% in BA 20 ([^{125}I]αBGT binding — BA 36 delusional misidentification: 2.2 ± 0.6 and no delusional misidentification: 3.1 ± 1.0 fmol/mg tissue equivalent; BA 20 delusional misidentification: 2.4 ± 0.9 and no delusional misidentification: 3.3 ± 1.0 fmol/mg tissue equivalent). No significant differences were seen for [^{3}H]epibatidine binding and groups of patients with or without delusional misidentification.

Table 4
Comparison of nicotinic receptor binding in temporal cortex between DLB cases with and without visual hallucinations, auditory hallucinations and delusional misidentification

	Visual hallucinations	No visual hallucinations	Statistical evaluation
[^{125}I]αBungarotoxin binding			
BA 20	2.2 ± 0.79 (17) [↓31%]	3.2 ± 0.94 (5)	t = 2.41, P = .025
BA 36	2.0 ± 0.66 (17) [↓31%]	2.9 ± 1.0 (5)	t = 2.56, P = .018
[^{3}H]Epibatidine binding			
BA 20	2.6 ± 0.87 (17) [↑4%]	2.5 ± 0.43 (4)	t = 0.25, P = .81
BA 36	1.9 ± 1.09 (17) [↑19%]	1.6 ± 0.41 (4)	t = 0.57, P = .58
	Auditory hallucinations	No auditory hallucinations	Statistical evaluation
[^{125}I]αBungarotoxin binding			
BA 20	2.0 ± 0.53 (10) [↓26%]	2.7 ± 1.02 (13)	t = 1.98, P = .061
BA 36	1.9 ± 0.50 (10) [↓21%]	2.4 ± 0.96 (13)	t = 1.60, P = .13
[^{3}H]Epibatidine binding			
BA 20	2.7 ± 0.81 (10) [↑8%]	2.5 ± 0.82 (12)	t = 0.33, P = .75
BA 36	1.9 ± 1.09 (18) [↑19%]	1.6 ± 0.41 (4)	t = 0.56, P = .65
	Delusional misidentification	No delusional misidentification	Statistical evaluation
[^{125}I]αBungarotoxin binding			
BA 20	2.1 ± 0.71 (15) [↓30%]	3.0 ± 1.03 (7)	t = 2.46, P = .023
BA 36	1.9 ± 0.58 (15) [↓30%]	2.7 ± 1.05 (7)	t = 2.41, P = .025
[^{3}H]Epibatidine binding			
BA 20	2.6 ± 0.92 (15) [↑4%]	2.5 ± 0.54 (6)	t = 0.45, P = .66
BA 36	1.9 ± 1.16 (15) [↓5%]	2.0 ± 0.60 (6)	t = 0.25, P = .81

Values are means ± S.D. in femtomoles per milligram tissue; the numbers in parenthesis are the number of determinations and those in square brackets indicate the mean percent difference in DLB cases with the psychotic feature compared to those without.

3.4. Evaluations using linear regression

In the linear regression analysis focussing upon [^{125}I]αBGT binding in BA 36, there was a significant association between visual hallucinations and reduction of αBGT binding (t = 2.5 P = .02), but neither auditory hallucinations (t = 0.59 P = .56) nor delusional misidentification (t = 0.97 P = .35) were entered into the equation. In contrast, the linear regression focusing upon [^{125}I]αBGT binding in BA 20 indicated that delusional misidentification was significantly associated with reduced [^{125}I]αBGT binding (t = 2.5 P = .02), but neither visual hallucinations (t = 0.75 P = .46) nor auditory hallucinations (t = 0.97 P = .35) were entered into the equation. Medication usage, including neuroleptic medication, and age were not entered into the equation for either analysis. MMSE appeared to have a weak effect on changes in [^{125}I]αBGT binding (t = − 2.151, P = .05 in BA 20 and t = − 1.979, P = .063 in BA 36).

3.5. Separate comparison between controls and DLB patients with and without visual hallucinations

DLB patients with visual hallucinations (n = 18) had similar levels of [^{125}I]αBGT binding in the temporal cortex to elderly controls (BA 36: 2.0 ± 0.7 vs. 1.9 ± 0.8 fmol/mg tissue equivalent; BA 20: 2.2 ± 0.8 vs. 2.0 ± 0.5 fmol/mg tissue equivalent). In contrast, nonhallucinators (n = 5) tended to have higher levels of [^{125}I]αBGT binding (BA 36: 2.9 ± 1.0 vs. 1.9 ± 0.8 fmol/mg tissue equivalent, t = 1.9 P = .06; BA 20: 3.2 ± 0.9 vs. 2.0 ± 0.5 fmol/mg tissue equivalent, t = 2.5 P < .05; Tables 3 and 4).

4. Discussion

The current study, which employed standardised prospective clinical assessments at regular intervals until death, has a methodological advantage over previous retrospective reports examining the neurochemical correlates of psychosis in dementia. This is the first study to evaluate potential associations between nAChRs and psychosis and the first to assess the neurochemical associations of delusional misidentification and auditory hallucinations. The number of patients investigated compares favourably with other clinico-neurochemical studies; however, the sample is too small to provide definitive evidence based on multiple comparisons. Nevertheless, the present results, which should be considered preliminary, indicate a potentially important association between lower nAChRs of the α7 subtype in DLB and psychotic symptoms in the visual modality.

Although there was no evidence of a change in cortical α7 containing nAChRs, determined by [^{125}I]αBGT, in the overall cohort of DLB cases compared to elderly controls,

a significant association was evident between reduced [^{125}I]αBGT binding in DLB patients with hallucinations or delusional misidentification compared to those without these psychotic symptoms. Further evaluation indicated that levels of [^{125}I]αBGT binding were similar in DLB cases with hallucinations and elderly controls, but were significantly elevated in nonhallucinators compared to control cases. It is possible that this apparent up-regulation of α7 nAChRs in DLB cases not experiencing hallucinations may be the result of a compensatory mechanism. It has been previously reported that variable ability of DLB patients to up-regulate dopamine D2 receptors may be associated with severe neuroleptic sensitivity: cases that were particularly susceptible to the adverse effects of neuroleptics were those with an apparent relative inability to up-regulate D2 receptors (Piggott et al., 1998). That α7 receptors may participate in compensatory up-regulation in age-related neurodegenerative disorders is suggested by the higher levels of α7 mRNA (although not protein) expression in the hippocampus of AD cases compared to age-matched controls reported in one study (Hellstrom-Lindahl et al., 1999). If the relatively reduced [^{125}I]αBGT binding in DLB cases with visual hallucinations was simply a reflection of the greater loss of cortical cholinergic innervation in this subgroup of cases, consistent with the presence of these receptors on such terminals (Sugaya et al., 1991), a significant loss in this receptor would have also been expected in all DLB cases compared with controls in parallel with the cortical choline acetyltransferase deficit (Ballard et al., 2000).

In contrast, there was a significant reduction of receptors with high affinity for epibatidine in DLB cases compared to controls, but there was no association, however, between this receptor subtype and any of the psychotic phenomena. Although the observed loss of cortical [^{3}H]epibatidine binding in DLB is consistent with the findings of Reid et al. (2000), the reduction of [^{125}I]αBGT BGT binding that was observed by this group in the frontal cortex in DLB, was not paralleled by the present observations in the temporal cortex. Differences in receptor expression in diverse cortical areas may reflect variable associations with different clinical symptoms and neuropathological components of the disease, and further evaluation of cohorts of patients with prospective psychiatric and psychometric assessment may be worthwhile. It may be that reduced cortical epibatidine binding in DLB is related to another common clinical symptom in DLB, such as fluctuations in consciousness.

The current observations indicate that specific alterations in the α7 nAChRs may be a substrate of visual hallucinations. Consistent with this are the findings that in schizophrenia, another disease characterised by psychotic symptoms, the α7 nicotinic receptor is implicated on the basis of genetic linkage data (Freedman et al., 1997; Leonard et al., 1996) and also reduced receptors and α7 protein expression in autopsy tissue (Freedman et al., 1995; Court et al., 1999; Guan et al., 1999; Durany et al., 2000). The current work also indicates the possibility of the α7 subunit being a novel potential target for the pharmacological treatment of hallucinations in dementia.

The data regarding delusional misidentification are more difficult to interpret. The degree of overlap between visual hallucinations and delusional misidentification was high. Förstl et al. (1991) have previously highlighted the link between delusional misidentification and visual agnosia, emphasising the importance of visual processing as a risk factor. A large degree of overlap and a shared chemical basis between visual hallucinations and delusional misidentification is therefore likely. The regression models did suggest that loss of [^{125}I]αBGT binding in BA 36 was predominantly associated with visual hallucinations, whereas loss of [^{125}I]αBGT in BA 20 was associated with delusional misidentification. Whilst it is plausible that the two symptoms, both dependent upon visual processing, may be related to loss of α7 nicotinic receptors in different areas of the visual processing system, further studies are needed focussing upon patients with only delusional misidentification or visual hallucinations, although this is not straightforward as such cases are unusual.

In DLB, but not AD, the majority of patients with visual hallucinations also experience the hallucinated figures in the auditory modality (Ballard et al., 1997b). It is, therefore, likely that in DLB auditory hallucinations are largely an epiphenomena of visual hallucinations.

Visual hallucinations only occurred in patients taking L-dopa, but not all patients taking L-dopa had visual hallucinations. Although the numbers were too small within the L-dopa-treated cases for formal comparison, [^{125}I]αBGT binding still tended to be reduced in hallucinating patients not taking L-dopa. Whilst this would be consistent with the possibility that in DLB L-dopa may play an adjunctive role in increasing vulnerability to visual hallucinations, it also indicates that lower α7 nicotinic receptor expression in the temporal cortex remains a predisposition to visual hallucinations in these individuals.

5. Conclusion

The results of the present study suggest that visual hallucinations and delusional misidentification may be associated with lower α7 nicotinic receptor binding in the temporal cortex of individuals with DLB. This supports previous work linking hallucinations to cholinergic deficits in DLB patients and is consistent with a potential link between psychosis in DLB and schizophrenia, where abnormalities of the α7 receptor have been reported. Further work is warranted on larger groups of patients to enable comparisons to be made between those with more and less frequent visual hallucinations and delusional misidentification, and to focus upon the possible additive effects of neurochemical disposition and L-dopa therapy on the genesis of visual hallucinations. Given the recent development

of α7 specific drugs, there is the possibility of novel treatments for hallucinations based upon manipulation of the nicotinic system.

Acknowledgments

The study was completed with the support of an MRC programme grant.

References

Ballard CG, Saad K, Patel A, Gahir M, Solis M, Coope B, Wilcock G. The prevalence and phenomenology of psychotic symptoms in dementia sufferers. Int J Geriatr Psychiatry 1995;10:477–85.

Ballard C, McKeith I, Burn D, Harrison R, O'Brien J, Lowery K, Campbell M, Perry R, Ince P. The UPDRS scale as a means of identifying extrapyramidal signs in patients suffering from dementia with Lewy bodies. Acta Neurol Scand 1997a;96:366–71.

Ballard CG, O'Brien J, Coope B, Fairbairn A, Abid F, Wilcock G. A prospective study of psychotic symptoms in dementia sufferers. Int Psychogeriatr 1997b;9:57–64.

Ballard CG, Grace JB, McKeith IG, Holmes C. Neuroleptic sensitivity in dementia with Lewy bodies and Alzheimer's disease. Lancet 1998;351: 1032–3.

Ballard C, Holmes C, McKeith I, Neill D, O'Brien J, Perry E, Ince P, Perry R. Psychiatric morbidity in dementia with Lewy bodies: a prospective clinical and neuropathological comparative study with Alzheimer's disease. Am J Psychiatry 1999;156:1039–145.

Ballard C, Piggott M, Johnson M, Cairns N, Perry R, McKeith I, Jaros E, O'Brien J, Holmes C, Perry EK. Delusions associated with elevated muscarinic binding in dementia with Lewy bodies. Ann Neurol 2000; 48:868–76.

Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 1991;82:239–59.

Burns A, Jacoby R, Levy R. Psychiatric phenomena in Alzheimer's. Br J Psychiatry 1990;157:72–96.

Chui HC, Lyness SA, Sobel E, Schneider LS. Extrapyramidal signs and psychiatric symptoms predict faster cognitive decline in Alzheimer's Disease. Arch Neurol 1994;51:676–81.

Clarke PBS. The fall and rise of neuronal α-bungarotoxin binding proteins. Trends Pharmacol Sci 1992;13:407–13.

Court JA, Lloyd S, Thomas N, Piggott MA, Marshall EF, Morris CM, Lamb H, Perry RH, Johnson M, Perry EK. Dopamine and nicotinic receptor binding and the levels of dopamine and homovanillic acid in human brain related to tobacco use. Neuroscience 1998;87:63–78.

Court J, Spurden D, Lloyd S, McKeith I, Ballard C, Cairns N, Perry R, Perry E. Neuronal nicotinic receptors in dementia with Lewy bodies and schizophrenia: αbungarotoxin and nicotine binding in the thalamus. J Neurochem 1999;73:1590–7.

Court JA, Piggott MA, Lloyd S, Cookson N, Ballard CG, McKeith IG, Perry RH, Perry EK. Nicotine binding in the human striatum: elevation in schizophrenia and reductions in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease and in relation to neuroleptic medication. Neuroscience 2000;98:79–87.

Cummings JL, Benson DF. Dementia: definition, prevalence, classification and approach to diagnosis. In: Cummings JL, Benson DF, editors. Dementia: a clinical approach. Boston: Butterworth-Heineman, 1991. pp. 1–17.

Devanand DP, Miller L, Richards M, Marder K, Bell K, Mayeux R, Stern Y. The Columbia University Scale for Psychopathology in Alzheimer's disease. Arch Neurol 1992;49:371–6.

Deimling GT, Bass DM. Symptoms of mental impairment among elderly adults and their effects on family caregivers. J Gerontol 1986;41: 778–84.

Dewey ME, Copeland JRM, Lobo A, Saz P, Dia SL. Computerised diagnosis from a standardised history schedule: a preliminary communication about the organic section of the HAS–AGECAT system. Int J Geriatr Psychiatry 1992;7:443–6.

Drevets WC, Rubin EH. Psychotic symptoms and the longitudinal course of senile dementia of the Alzheimer type. Biol Psychiatry 1989;25:39–48.

Durany N, Zöchling R, Boissl KW, Paulus W, Ransmayr G, Tatschner T, Danielczyk W, Jellinger K, Deckert J, Riederer P. Human post-mortem striatal α4β2 nicotinic acetylcholine receptor density in schizophrenia and Parkinson's syndrome. Neurosci Lett 2000;287:109–12.

Ellerman JM, Siegal JD, Strupp JP, Ebner TJ, Ugurbil K. Activation of visuomotor systems during visually guided movement: a functional MRI study. J Magn Reson 1998;131:272–85.

Fergusson E, Howard R. Donepezil for the treatment of psychosis in dementia with Lewy bodies. Int J Geriatr Psychiatry 2000;15:280–1.

Förstl H, Almeida OP, Owen AM, Burns A, Howard R. Psychiatric, neurological and medical aspects of misidentification syndromes: a review of 260 cases. Psychol Med 1991;2:905–10.

Förstl H, Besthorn C, Geiger-Kabisch C, Sattel H, Schreiter-Gasser U. Psychotic features and the course of Alzheimer's disease: relationship to cognitive electroencephalographic and computerised tomography. Acta Psychiatr Scand 1993;87:395–9.

Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. Biol Psychiatry 1995;38:22–33.

Freedman R, Conn H, Myles-Worseley M, Orr-Urteger A, Olincy A, Davis A, Polymeropulos M, Holik J, Hopkin JJR, Waldo M, Reimherr F, Wender P, Yaw J, Young D, Breese C, Adams C, Patterson D, Adler L, Kruglyak L, Leonard S, Byerley W. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. Proc Natl Acad Sci USA 1997;94:587–92.

Gray A, Fenn P. Alzheimer's disease: the burden of illness in England. Health Trends 1993;20:31–7.

Gilley DW, Whalen ME, Wilson RS, Bennett DA. Hallucinations and associated factors in Alzheimer's disease. J Neuropsychiatry 1991;3:371–6.

Goetz CG, Pappert EJ, Blasucci LM, Stebbins GT, Ling ZD, Nora MV, Carvey PM. Intravenous levodopa in hallucinating Parkinson's disease patients: high dose challenge does not precipitate hallucinations. Neurology 1998;50:515–7.

Guan ZZ, Zhang X, Blennow K, Nordberg A. Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. NeuroReport 1999;10:1779–82.

Haeske-Dewick HC. Hallucinations in Parkinson's disease: characteristics and associated clinical features. Int J Geriatr Psychiatry 1995;10: 487–95.

Haupt M, Romero B, Kurz A. Delusions and hallucinations in Alzheimer's disease. Int J Geriatr Psychiatry 1996;11:965–72.

Hellstrom-Lindahl E, Mousavi M, Zhang X, Ravid R, Nordberg A. Regional distribution of nicotinic receptor subunit mRNAs in human brain: comparison between Alzheimer and normal brain. Mol Brain Res 1999;66:94–103.

Hutchinson M, Fazzini E. Cholinesterase inhibition in Parkinson's disease. J Neurol Neurosurg Psychiatry 1996;61:324–5.

Kaufer D, Shea C, Macknight C, Rockwood R. Donepezil for treatment of dementia with Lewy bodies: a case series of nine patients. Int Psychogeriatr 1998;10:229–39.

Leonard S, Adams C, Breese C, Adler L, Bickford P, Byerley W, Coon H, Griffith J, Miller C, Myles-Worsley M, Nagamoto H, Rollins Y, Stevens K, Waldo M, Freedman R. Nicotinic receptor function in schizophrenia. Schizophr Bull 1996;22:431–45.

Levy R, Eagger S, Griffiths M. Lewy bodies and response to tacrine. Lancet 1994;343:176.

McKeith IG, Fairbairn A, Perry R, Thompson P, Perry E. Neuroleptic sensitivity in patients with senile dementia of Lewy body type. Br Med J 1992;305:673–8.

McKeith IG, Ballard CG, Harrison RWS. Neuroleptic sensitivity to risperidone in Lewy body dementia. Lancet 1995;346:699.

McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, Salmon DP, Lowe J, Mirra SS, Byrne EJ, Lennox G, Quinn NP, Edwardson JA, Ince PG, Bergeron C, Burns A, Miller BL, Lovestone S, Collerton D, Jansen ENH, Ballard C, de Vos RAI, Wilcock GK, Jellinger KA, Perry RH. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB). Neurology 1996;47:1113–24.

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L, Participating CERAD Neuropathologists. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD): II. Standardisation of the neuropathological assessment of Alzheimer's disease. Neurology 1991;41:479–86.

Morris JC, Cyrus PA, Orazem J, Mas J, Bieber F, Ruzicka BB, Gulanski B. Metrifonate benefits cognitive, behavioural and global function in patients with Alzheimer's disease. Neurology 1998;50:1222–30.

Paterson D, Nordberg A. Neuronal nicotinic receptors in the human brain. Prog Neurobiol 2000;61:75–111.

Pearlin LI, Mullen JT, Semple SJ, Skaff MM. Caregiving and the stress process: an overview of concepts and their measures. Gerontology 1990;30:583–94.

Perry EK, Perry RH. Acetylcholine and hallucinations: disease-related compared to drug-induced alterations in human consciousness. Brain Cognit 1995;28:240–58.

Perry EK, Marshall E, Smith C, Jabeen S, Cheng AV, Perry RH. Evidence of a monoaminergic; cholinergic imbalance related to visual hallucinations in Lewy body dementia. J Neurochem 1990a;55:1454–6.

Perry EK, Smith CJ, Court JA, Perry RH. Cholinergic nicotinic and muscarinic receptors in dementia of Alzheimer, Parkinson and Lewy body types. J Neural Transm 1990b;2:149–58.

Perry RH, Irving D, Blessed G, Fairbairn A, Perry EK. A clinically and pathologically distinct form of Lewy body dementia in the elderly. J Neurol Sci 1990c;95:119–39.

Perry EK, Morris CM, Court JA, Cheng A, Fairbairn AF, McKeith IM, Irving D, Brown A, Perry RH. Alteration in nicotine binding sites in Parkinson's disease, Lewy body dementia and Alzheimer's disease: possible index of early neuropathology. Neuroscience 1995;64:385–95.

Piggott MA, Perry EK, Marshall EF, McKeith IG, Johnson M, Melrose HL, Court JA, Lloyd S, Fairbairn A, Brown A, Thompson P, Perry RH. Nigrostriatal dopaminergic activities in dementia with Lewy bodies in relation to neuroleptic sensitivity: comparisons with Parkinson's disease. Biol Psychiatry 1998;44:765–74.

Rabins PV, Mace NL, Lucas MJ. The impact of dementia on the family. J Am Med Soc 1982;248:333–5.

Reid RT, Sabbagh MN, Corey-Bloom J, Tirababoschi P, Thal LJ. Nicotinic receptor losses in dementia with Lewy bodies: comparisons with Alzheimer's disease. Neurobiol Aging 2000;21:741–6.

Rockwell E, Jackson E, Vilke G, Jeste DV. A study of delusions in a large cohort of Alzheimer's disease patients. Am J Psychiatry 1994;2:157–64.

Rosen J, Zubenko GS. Emergence of psychosis and depression in the longitudinal evaluation of Alzheimer's disease. Biol Psychiatry 1991; 29:2224–32.

Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrick H, Vermas Goddard R. CAMDEX: a standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986;149:698–709.

Sugaya K, Downen M, Giacobini E. Nucleus basalis lesions decrease α and κ bungarotoxins binding in rat cortex. NeuroReport 1991;2:177–80.

Shea C, MacKnight C, Rockwood K. Donepezil for treatment of dementia with Lewy bodies: a case series of nine patients. Int Psychogeriatr 1998; 10:229–38.

Steele C, Rovner B, Chase GA, Folstein M. Psychiatric symptoms and nursing home placement of patients with Alzheimer's disease. Am J Psychiatry 1990;147:1049–51.

Zoldan J, Friedberg G, Weisman A, Melamed E. Ondansatron, a 5-HT_3 antagonist for visual hallucinations and paranoid delusional disorder associated with L-dopa therapy in advanced Parkinson's disease. Adv Neurol 1996;69:541–4.

Zoli M, Lena C, Picciotto M, Changeux J-P. Identification of four classes of brain nicotinic receptors using beta2 mutant mice. J Neurosci 1998;18: 4461–72.

Zubenko GS, Moossy J, Martinez J, Rao G, Claassen D, Rosen J, Kopp U. Neuropathologic and neurochemical correlates of psychosis in primary dementia. Arch Neurol 1991;48:619–24.