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Alzheimer's Disease Is Associated With a Selective Increase in α7 Nicotinic Acetylcholine Receptor Immunoreactivity in Astrocytes

THANASAK TEAKTONG,[1] ALISON GRAHAM,[1] JENNIFER COURT,[1] ROBERT PERRY,[2] EVELYN JAROS,[2] MARY JOHNSON,[1] ROS HALL,[1] AND ELAINE PERRY[1]*

[1]*MRC Building, Centre Development in Clinical Brain Aging, Newcastle General Hospital, Newcastle Upon Tyne, U.K.*

[2]*Department of Neuropathology, Newcastle General Hospital, Newcastle Upon Tyne, U.K.*

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ABSTRACT Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) are common forms of dementia in the elderly associated with cholinergic dysfunction, including reductions in nicotinic acetylcholine receptors (nAChRs). In AD, astrocytes are implicated in the formation of senile plaques, one of the core pathological features. Using immunohistochemistry, we have investigated astrocytic expression of the two major nicotinic receptor α subunits in the human hippocampus and entorhinal cortex. α7, but not α4, subunit immunoreactivity was associated with astrocytes. An increase in the proportion of astrocytes expressing α7 immunoreactivity was observed in AD compared with age-matched controls. A similar increase was not evident in DLB. Elevated α7 nAChRs on astrocytes in AD may contribute to alterations in calcium homeostasis and nitric oxide production, which in turn could affect β-amyloid–mediated inflammatory processes in AD. *GLIA 41:207–211, 2003.* © 2003 Wiley-Liss, Inc.

Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) (McKeith et al., 1996; Winblad et al., 2001) together account for more than half of the dementia in the elderly. Both disorders are affected by pathology associated with impaired cholinergic function, including deficits in brain nicotinic acetylcholine receptors (nAChRs) in cortical and other brain areas (Court et al., 2001). nAChRs are ligand-gated cation channels that are expressed not only on neurons, but also on astrocytes where they participate in calcium signaling (Sharma and Vijayaraghavan, 2001). Astrocytes are likely to be involved in the pathology of AD; they surround senile plaques (Kato et al., 1998), appear to promote amyloid plaque maturation (Terai et al., 2001; Wegiel et al., 2001), and can induce neurotoxicity cascades involving many inflammatory mediators (Sutton et al., 1999; Combs et al., 2000). DLB is characterized by α-synuclein–positive Lewy bodies and neurites in the cerebral cortex and brain stem and with cortical senile plaques to a lesser degree than in AD (Ince et al., 1991). The present study explores the possible involvement of nicotinic receptors in astrocytosis in AD and makes comparison with DLB. The expression of the two major nAChR α subunits (α4 and α7) (Gotti et al., 1997) was investigated in the hippocampus and entorhinal cortex using immunohistochemistry.

Autopsy samples of temporal cortex were obtained from six cases of AD (age, 83.7 ± 2.7 years), six cases of DLB (age, 76.5 ± 3.1 years), and four age-matched controls (age, 82.5 ± 9.5 years). Mean postmortem de-

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*Correspondence to: Elaine Perry, MRC Building, Centre Development in Clinical Brain Aging, Newcastle General Hospital, Westgate Road, Newcastle Upon Tyne, NE4 6BE, U.K. E-mail: e.k.perry@ncl.ac.uk

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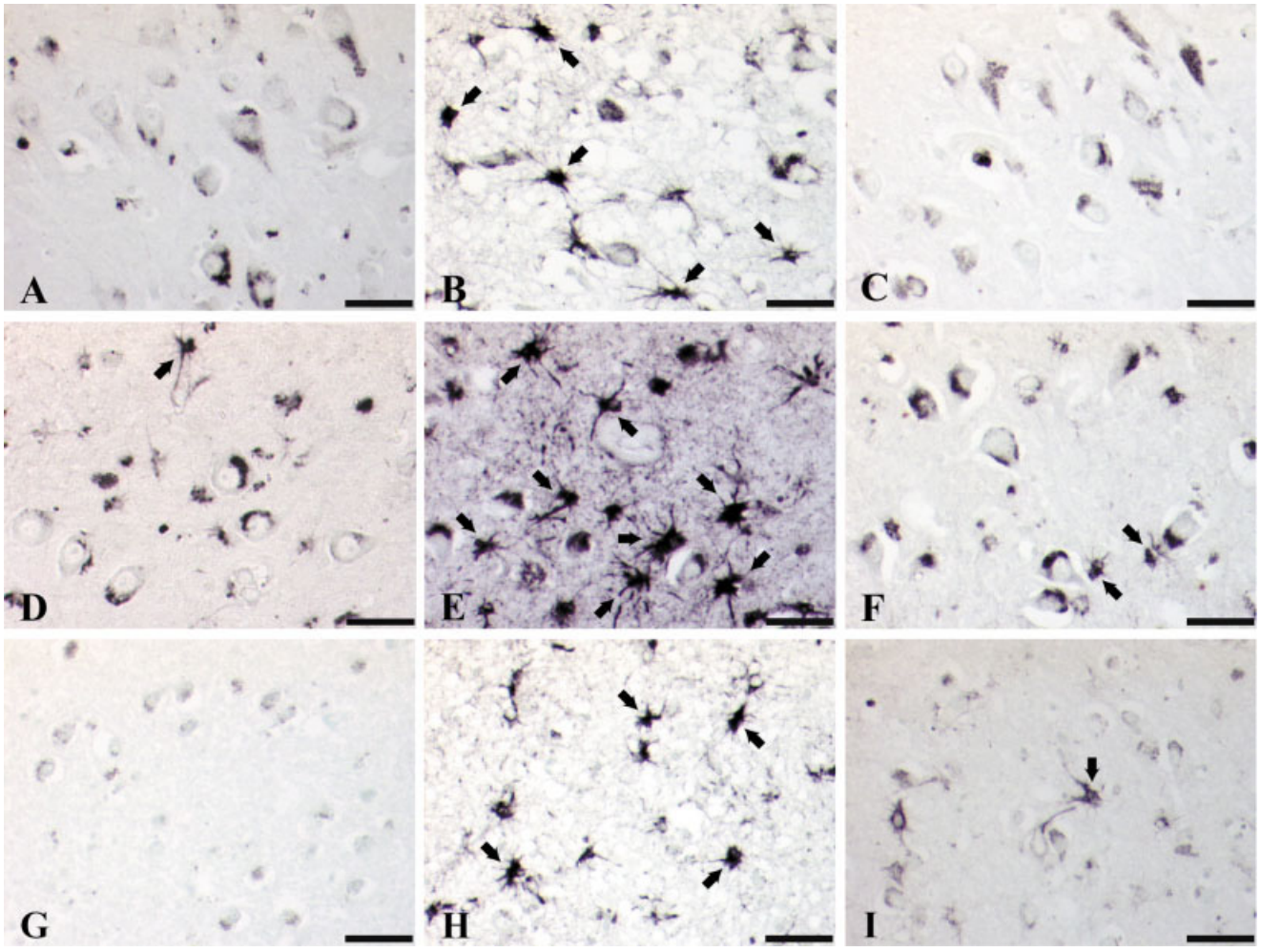

Fig. 1. α7-immunoreactive astrocytes in CA1 (**A–C**), CA4 (**D–F**), and entorhinal cortex layer 3 (**G–I**) of control (A, D, and G), Alzheimer (B, E, and H), and DLB (C, F, and I) cases. α7-immunoreactive astrocytes were found in all groups but were significantly increased in AD cases compared with controls. Arrows indicate α7-immunoreactive astrocytes. Scale bars, 20 μm.[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

lays of control, AD, and DLB cases were 46.0 ± 18.9, 58.8 ± 24.5, and 41.2 ± 33.4 h, respectively. We have previously shown that this range of postmortem delay does not affect nAChR immunohistochemistry (Graham et al., 2002). None of the controls smoked tobacco or had any history of a neurological or psychiatric disease or significant neuropathological abnormality. AD and DLB were diagnosed according to neuropathological examination and clinical diagnostic criteria (Perry et al., 1990; McKeith et al., 1996). Ten μm thick formalin-fixed paraffin-embedded sections were immunostained with monoclonal antibodies against the α4 (mAb 299, 1:8,000; Cambridge Bioscience, Cambridge, U.K.) and α7 nAChR subunits (mAb 306, 1:4,000; Cambridge Bioscience) and with polyclonal antiglial fibrillary acidic protein (GFAP; 1:4,000; Dako, Cambridgeshire, U.K.) as a marker of astrocytes, using the Vectastain Elite kit method. The specificity of the monoclonal antibodies has been established elsewhere; for mAb 299, Whiting and Lindstrom (1988), Peng et al. (1994), and Schroder et al. (2001); for mAb 306, Schoepfer et al. (1990), McLane et al. (1992), Burghaus et al. (2000). For double labeling of α7 and GFAP, antibodies were applied sequentially using the anti-α7 followed by anti-GFAP. Controls from which primary antibodies were omitted showed no immunoreactivity. Controls in which the anti-GFAP was replaced by rabbit IgG confirmed no cross-reactivity between the two secondary antisera. The percentage of α7-immunoreactive astrocytes was assessed in double-stained sections using a light microscope at 20 × magnification in CA1, CA4, and entorhinal cortex. The entorhinal cortex was subdivided into three layers: layer 1 corresponded to the superficial acellular layer, layer 2 the layer of islands of small pyramidal cells, and layer 3 the layers of medium and large pyramidal cells extending below layer 2 to the white matter. Group differences were evaluated using the Mann-Whitney test.

α7 immunoreactivity was present on astrocytes in all three groups (Figs. 1 and 2A, C, and E). In controls, the

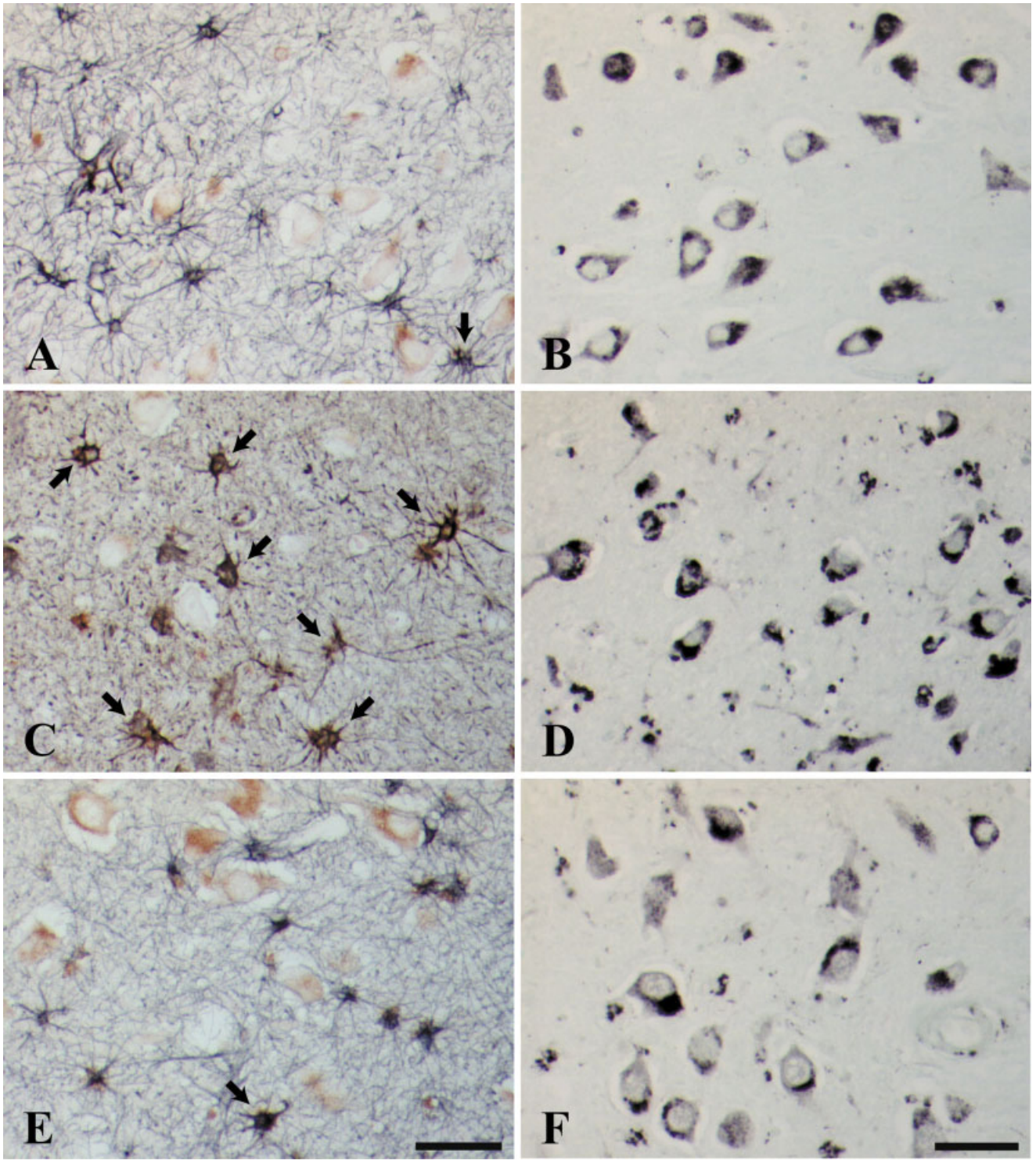

Fig. 2. Immunohistochemistry of α7 nAChR and GFAP double labeling (**A**, **C**, and **E**) and α4 nAChR (**B**, **D**, and **F**) in CA4 of control (A and B), Alzheimer (C and D), and DLB (E and F) cases. Red and blue/gray stainings show α7 and GFAP immunoreactivity, respectively. Both α7 and GFAP immunoreactivity were increased in Alzheimer's disease but not DLB compared with controls. Arrows indicate double-immunolabeled astrocytes in control, AD, and DLB. No α4 immunoreactive astrocytes were found in all three groups. Scale bars, 20 μm.

percentage of α7-labeled astrocytes varied between 0% and 54% depending on area, being low in CA1 and layer 3 of the entorhinal cortex and highest in CA4 and entorhinal cortex layer 1 (which contained the pial surface; Table 1), possibly reflecting regional differences in astrocytic function or activation. In DLB, a similar pattern was observed in controls. However, in AD, the percentage of α7-immunoreactive astrocytes

TABLE 1. Percentage of α7 and GFAP Double-Labeled Compared With the Total Number of GFAP–Labeled Astrocytes

Brain area	Control	AD	DLB
Pyramidal cell layer			
CA1	12.5 ± 25	61.1 ± 9.6[a]	11.1 ± 19.2
CA4	37.5 ± 8.4	80.6 ± 17.3[b]	25.0 ± 43.3
Entorhinal cortex			
Layer 1	54.2 ± 8.4	100.0 ± 0.0[b]	77.8 ± 38.5
Layer 2	0.0 ± 0.0	83.3 ± 28.9[b]	16.7 ± 28.9
Layer 3	20.8 ± 25.0	61.1 ± 9.6[a]	22.2 ± 19.2

[a]$P < 0.05$.
[b]$P < 0.01$, compared to control.

was markedly greater in the hippocampus and entorhinal cortex compared with control and DLB cases. In contrast to the large numbers of α7-reactive astrocytes found in the hippocampus and entorhinal cortex in AD, no α4-immunoreactive astrocytes were observed in these areas in AD, controls, or DLB (Fig. 2B, D, and F). As expected, astrocytes assessed by GFAP immunoreactivity were more numerous in most subfields of the hippocampal formation of AD compared with both DLB and control groups; however, in CA4 (Fig. 2A, C, and E), astrocyte density was similar between groups.

Wevers et al. (1999) also noted α7 expression on cells with astrocytic profiles in the frontal cortex in AD, some associated with amyloid plaques, but not in age-matched controls. That α7 expression on astrocytes is greater in both controls and AD cases in hippocampus and entorhinal cortex than the neocortex is likely to reflect the greater age-related pathology of the archicortex. The upregulation of mRNA for α7 observed by Hellstrom-Lindahl et al. (1999) in hippocampus but not in the temporal cortex or cerebellum in AD is consistent with such a mechanism. The present findings contrast with the net reduction in α7 protein expression and α-bungarotoxin binding previously observed in some studies in the hippocampus in AD (Court et al., 2001). Nevertheless, there was no relationship between increased α7 astrocytic immunoreactivity in entorhinal cortex and the severity of β-amyloidosis as indicated by the numbers of neocortical plaques stained histologically (Perry et al., 1990) in either AD or DLB ($r = 0.098$, $P = 0.854$ in AD; $r = -0.099$, $P = 0.852$ in DLB).

The increased expression of α7 on astrocytes in AD could be in response to the reduction in acetylcholine release. However, if this were the case, a similar increase in astrocytic α7 expression would be expected to occur in DLB in which cholinergic innervation is equally reduced (Tiraboschi et al., 2000). Beta-amyloid induces many proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) (Sutton et al., 1999; Szczepanik et al., 2001), which can induce nitric oxide production and release from astrocytes (Casamenti et al., 1999; Pazmany et al., 1999). Astrocytic expression of the calcium-dependent NOS enzymes is reported to be increased in both archi- and neocortex in AD (de la Monte and Bloch, 1997; Simic et al., 2000). However, if β-amyloid accumulation is the sole mechanism whereby astrocytic α7 expression is upregulated in AD, it is surprising that this does not also occur in DLB. It is possible that the difference observed between AD and DLB at least in part reflects distinct pathological processes in the two disorders.

Stimulation of α7 nAChRs on astrocytes has been shown to increase intracellular calcium released from intracellular stores (Sharma and Vijayaraghavan, 2001), hence elevated expression of α7 nAChR on astrocytes may lead to abnormally high levels of intracellular calcium, which might contribute to a number of inflammatory cascades.

Further studies are required to explore the potential mechanisms of astrocytic α7 nAChR upregulation in AD and the contribution that this might make to AD pathology.

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