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Polyadenylated Messenger RNA in Paired Helical Filament-Immunoreactive Neurons in Alzheimer Disease

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Summary

An antibody raised against isolated paired helical filaments (PHF) was used to identify tangle-bearing (PHF⁺) neurons in autopsy brain tissue from six Alzheimer disease (AD) patients and six age-matched controls (AMC). A comparison of the levels of polyadenylated messenger RNA [poly(A)⁺ mRNA] in PHF⁺ and PHF⁻ neurons of similar cross-sectional area in temporal and parietal lobe and cerebellum from four AD and four AMC brains was made by analysis of in situ hybridization of [³H] polyuridylylate [poly(U)] to intracellular poly(A)⁺ mRNA. In PHF⁺ neurons, the level of poly(A)⁺ mRNA was approximately two-thirds that in similar-sized PHF⁻ neurons in either AD or AMC. The level of poly(A)⁺ mRNA in PHF⁻ neurons in regions of the brain that have more of the histopathologically defined effects in AD was similar to that in regions with less effects.

Keywords

Paired helical filament; Tangle-bearing neurons; Alzheimer disease; Polyadenylated messenger RNA

The intracellular accumulation of neurofibrillary tangles in certain cortical and subcortical neurons and the presence of neuritic plaques with β -amyloid cores are characteristic of the neuropathology of Alzheimer disease (AD) (Khachaturian, 1985). Morphologically similar neural lesions appear with age, in older individuals with Down syndrome (Ellis et al., 1974; Ball, 1977; Wisniewski et al., 1985; Mann, 1988; Rumble et al., 1989; Woody et al., 1989), and certain other neurodegenerative diseases (Wisniewski et al., 1979). Neurofibrillary tangles are composed of paired helical filaments (PHF) (Kidd, 1963; Wisniewski et al., 1976) and accumulate in the cytoplasm of neurons that eventually die, leaving only the tangles referred to as “ghost” tangles (Rasool et al., 1984). It seems obvious that the presence of PHF in a cell would have detrimental effects on its function, perhaps including protein synthesis. The purpose of the present study was to determine whether such effects include compromises in the levels of total mRNA in PHF-containing neurons.

One intracellular constituent affected by PHF accumulation is ribosomal RNA (rRNA). In many brain regions, the level of rRNA, assessed by nucleolar volume assay, is lower in tangle-bearing neurons than in tangle-free neurons of the same size class (Dayan and Ball, 1973; Mann et al., 1981a; Mann and Yates, 1981). The amount of total intracellular RNA in tangle-free neurons of prefrontal cortex in AD is similar to that in the same regions of brain from controls (Uemura and Hartman, 1978), whereas neurons with tangles have approximately one-half the RNA content of tangle-free neurons (Uemura and Hartman, 1979). Some reports show that only tangle-bearing neurons are depleted of RNA in AD (Dayan and Ball, 1973) while others report variable RNA losses (Mann et al., 1977). Using biopsy tissue, decreases in nucleolar volume in all cortical neurons have been demonstrated in AD (Mann et al., 1981a,b; Mann and Yates, 1981). The use of biopsy material rather than postmortem material may have eliminated potential artifacts that result in selective postmortem RNA degradation in AD tissue (Ilaria et al., 1985; Morrison et al., 1986, 1987a,b; Morrison, 1988).

Recently, the total RNA content within neurons in different brain regions of AD and AMC brains has been directly measured. Although the number of neurons containing tangles in different hippocampal regions does not correlate with the extent of regional RNA loss, azure B RNA staining shows that the total RNA content of hippocampal pyramidal cells is less in AD than in controls (Doebler et al., 1987a). Variable degrees of RNA loss have also been detected in neurons in regions other than hippocampus in AD brain (Doebler et al., 1987b). Using azure B staining, Neary and associates (1986) found less RNA in pyramidal cells of layers III (22%) and V (25%) in biopsies from AD cortex compared to control, indicating a strong correlation between neurofibrillary tangle frequency and lower levels of RNA. In addition, AD hippocampus cells containing lower levels of RNA contain Alz-50 immunoreactive product (Rhoads et al., 1987; Wolozin et al., 1986), suggesting that RNA levels decrease as a consequence of pathological processes in neurons in AD.

Total RNA can be isolated from postmortem AD and control brain, and the levels of specific mRNAs can be determined using either *in vitro* translation (Gilbert et al., 1981; Marotta et al., 1981; Morrison and Griffin, 1981; Morrison et al., 1983; Ilaria et al., 1985) or hybridization (Johnson et al., 1986). An overall decrease in total RNA and/or mRNA content has been reported in AD cortex (Sajdel-Sulkowska et al., 1983; Guillemette et al., 1986; Taylor et al., 1986, 1987; McLachlan et al., 1988), suggesting that the RNA content of several cell types in these regions in AD brain is decreased. We and others have reported that there is little or no difference in mRNA levels in corresponding regions of AD and control brain (Morrison et al., 1986, 1987a,b; Johnson and Finch, 1987; May et al., 1987; Morrison, 1988).

To characterize AD neuropathology at the molecular level and provide baseline information necessary for the measurement of the relative levels of specific mRNAs in different brain regions and cell types, we combined two techniques—immunohistochemistry, using an antibody raised against PHF, and *in situ* hybridization, using radiolabeled polyuridylylate, *i.e.*, [³H]poly(U). Two questions were addressed: (a) is the presence of PHF correlated with alterations in the levels of total hybridizable poly(A)⁺ mRNA in neurons of AD cortex; and (b) is the intracellular content of poly(A)⁺ mRNA in cortical and cerebellar neurons that do not contain PHF similar in AD and age-matched controls (AMQ)?

MATERIALS AND METHODS

Tissue Preparation

Brain tissue was collected at autopsy from six clinically diagnosed AD patients (mean age of 75 years, range of 63 to 86 years) and six AMC (mean age of 71 years, range of 56 to 94

years). Postmortem intervals of AD tissue ranged from 4 to 52 h (mean interval of 19.5 h) and of AMC from 12.5 to 83 h (mean interval of 27.7 h). The brains were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. The clinical diagnosis of AD (McKhann et al., 1984) was confirmed by neuropathological examination (Khachaturian, 1985) of silver-stained tissue sections (Sevier and Munger, 1965); the presence of neurofibrillary tangles in the hippocampus and seven or more neuritic plaques per 1.4 mm diameter 100× microscopic field in affected neocortex (Tomlinson et al., 1970) was confirmatory of AD. The number of plaques and tangles in all brain areas was negligible in each AMC. For immunohistochemical and in situ hybridization analyses, paraffin blocks containing superior temporal cortex, parietal cortex, and cerebellum were sectioned at 10 µm and mounted on gelatin-coated microscope slides. These brain regions were chosen because they have previously been shown to have different degrees of PHF accumulation (Hirano and Zimmerman, 1962; Hyman et al., 1984).

Combined In Situ Hybridization and Immunohistochemistry

Paraffin-embedded human brain tissue sections were rehydrated and processed for in situ hybridization followed by immunohistochemistry using modifications of procedures that we have previously described (Griffin, 1987, 1988).

In Situ Hybridization—Each section was permeabilized with 0.2 N HCl, 0.1% Triton X100, and 40 µg/ml of proteinase K, acetylated with acetic anhydride, hybridized with 0.9 µCi of [³H]poly(U) (New England Nuclear, Boston, MA) for 1 h at 50°C, treated with 50 µg of ribonuclease A in 4 × SSC (1 × SSC = 0.15 M NaCl; 0.05 M Na citrate, pH 8.0) for 30 min at 37°C to degrade single-stranded RNA, and washed in two changes, 20 min each, of 2 × SSC, containing 0.1% Triton X100, followed by 30 min in 1 × SSC at room temperature. As negative controls, adjacent sections were treated with 100 µg of ribonuclease A in 0.5 × SSC for 30 min at 37°C either before or after hybridization in order to degrade intracellular RNA.

Immunohistochemistry—Lyophilized whole rabbit serum obtained from animals that were immunized with a highly purified isolate of SDS-insoluble PHF (according to the method of Ihara et al., 1983) derived from AD brain (ICN, Lisle, IL, U.S.A.) was reconstituted with sterile distilled water and used to identify PHF-containing neurons. The immunoreactivity of this PHF antiserum can be neutralized by AD but not AMC brain extract (ICN, personal communication). Endogenous peroxidase activity was blocked in hybridized sections by treatment with 0.03% hydrogen peroxide (H₂O₂) in 100% methanol. Nonspecific binding of the secondary antibody was blocked by incubation with 20% nonimmune goat serum (NGS). The sections were incubated overnight, at room temperature, with PHF antibody (1:800). Subsequently, the sections were incubated with goat-anti-rabbit IgG (1:50) followed by rabbit peroxidase–antiperoxidase IgG (1:200) for 30 min each at room temperature. All antibodies were diluted in 2% NGS. The chromogen used to label the immunoreactive product was diaminobenzidine tetrahydrochloride (0.044% in ammonium acetate, pH 5.5, containing 0.003% H₂O₂; Sigma, St. Louis, MO, U.S.A.). As negative controls, adjacent tissue sections were similarly treated, except that nonimmune rabbit serum (ICN) replaced the primary antibody.

After hybridization and immunohistochemistry, the slides were dipped in Kodak NTB2 emulsion (Eastman Kodak, Rochester, NY, U.S.A.). The emulsion was exposed for 20 days at 4°C, developed in Kodak developer for 2 min at 17°C, fixed in Kodak fixer for 2 min at 17°C, and washed in cold, running water for 20 min. The sections were stained with hematoxylin and eosin.

Analyses

Autoradiographic grains were counted above PHF⁺ and PHF⁻ neurons of similar cross-sectional area (approximately 300 μm²) in tissue sections from the parietal and temporal lobe of AD and AMC brains; cerebellar Purkinje cells and cells in the deep cerebellar nuclei were also examined. Each cell chosen for analysis contained a visible nucleolus. To control for background, grain counts over cells in RNase pre- and posthybridization-treated sections were subtracted from experimental values. The mean number of autoradiographic grains per cell ± SEM was determined for 120 neurons in each brain. Significance was determined at $p < 0.05$ using the Student's *t* test.

RESULTS

Eight of the 12 brains examined yielded positive in situ hybridization results. Very few autoradiographic grains were detected over cells in brain tissue sections from two AD patients (aged 75 and 76 years, postmortem interval of 4 and 10 h, respectively) and two AMC (aged 56 and 69 years, postmortem interval of 83 and 14 h, respectively). The detection of fewer grains was likely due to RNA degradation in both AD and AMC brains. We have previously shown a similar percentage of degradation in RNA isolates from AD and AMC brains (Morrison et al., 1987*a,b*; Morrison, 1988). All brains analyzed, with the exception of one AD brain collected 52 h postmortem, had postmortem intervals ≤ 21 h; six were within 18 h.

PHF⁺ neurons were heavily distributed in superior temporal cortex in all AD brains examined. No PHF-immunoreactive cells were observed in the cerebella of AD brains or in the regions examined in the AMC brains. PHF⁺ neurons had fewer autoradiographic silver grains than did PHF⁻ neurons in all four AD brains examined, indicating that the levels of poly(A)⁺ mRNA hybridized in PHF⁺ neurons are reduced (Fig. 1). We have previously shown that the presence of intracellular immunoreactive product does not interfere with the detection of ³H-autoradiographic grains (Griffin, 1988).

Despite inherent variability in several parameters, including postmortem interval, the SEM grain counts over comparable neurons from AD and AMC did not vary from the mean by more than 5–10% (Figs. 2 and 3). The number of grains over PHF⁺ neurons (32.7 ± 2.1 , mean ± SEM) was approximately two-thirds that over PHF⁻ neurons (49.0 ± 2.5 ; $p = 0.05$) in AD cortex (Fig. 2). The number of grains over PHF⁻ neurons in more affected regions of AD brain, e.g., parietal cortex and temporal lobe, as well as in affected regions, e.g., cerebellum, was similar to the number over PHF⁻ neurons in AMC brain (52.5 ± 1.7) (Figs. 2 and 3).

DISCUSSION

The detection of fewer autoradiographic grains over PHF⁺ neurons compared to PHF⁻ neurons of comparable size in AD cortex following in situ hybridization of [³H]poly(U) indicates that intracellular accumulation of PHF is associated with a reduction (33%) in cellular poly(A)⁺ mRNA content. Although this reduction in poly(U)–poly(A) hybrids in PHF⁺ neurons could theoretically result from a reduction in length of the poly(A) attached to mRNAs (Morrison and Lingrel, 1976), our previous findings (Griffin et al., 1984, 1985) suggest that it is more likely the consequence of a reduction in the levels of total poly(A)⁺ mRNAs in neurons compromised by PHF. It follows that the levels of individual mRNAs in these cells are likely to be reduced by a similar magnitude, solely as a consequence of PHF accumulation (Palmer et al., 1988). The presence of other intracellular inclusions such as granulovacuolar degeneration and pigment may, in addition to their documented effect on

rRNA levels (Mann, 1978; Mann and Sinclair, 1978; Mann and Yates, 1979; Mann et al., 1978, 1982), contribute to decreased mRNA levels; their effects were not addressed here.

We conclude that the presence of neurons containing PHF does not compromise the total poly(A)⁺ mRNA content of neighboring neuronal populations since PHF⁻ cells in AD brains have levels of poly(A)⁺ mRNA similar to those in PHF⁻ neurons in AMC brains. The poly(A)⁺ mRNA levels in neurons in the cerebellar cortex and deep cerebellar nuclei (regions of AD brain that have relatively few plaques and tangles) were not different from the poly(A)⁺ RNA levels of similar cell types in AMC brains. Moreover, in temporal cortex, despite the presence of PHF⁺ neurons, there was no significant difference in poly(A)⁺ mRNA content in PHF⁻ neurons of similar size in AD patients and AMC. Because the total number of PHF⁺ neurons is small compared to the total number of neurons even in the most severely affected regions of AD brain, it is not surprising that the total levels of mRNAs representing all brain cell types would not be detectably reduced (Morrison et al., 1986; 1987a,b; Johnson and Finch, 1987; May et al., 1987; Morrison, 1988).

If specific mRNAs are preferentially synthesized in PHF⁺ neurons, then the overall cortical levels of those mRNAs and their translation products may be significantly reduced. Somatostatin-like immunoreactivity is decreased in cortex (Davies et al., 1980; Davies and Terry, 1981), particularly in temporal lobe (Rosser et al., 1980; Beal et al., 1986), where there is a high concentration of PHF-containing cells (Hyman et al., 1984). In addition, somatostatin immunoreactivity has been detected in plaques (Morrison et al., 1985; Armstrong et al., 1989) and in tangle-bearing cells in AD temporal lobe (Roberts et al., 1985), suggesting that degenerative processes occur in a population of somatostatin-containing neurons. The correlation, if any, between PHF content in neurons and a decrease in the levels of somatostatin mRNA is unknown, but a decrease in somatostatin mRNA in tangle-bearing cells could contribute to a reduction in somatostatin. Our findings of decreased total poly(A)⁺ mRNA in tangle-bearing neurons support the idea that the presence of PHF in a neuron decreases its capacity to synthesize specific gene products. This idea could be tested using in situ hybridization to determine the levels of a specific mRNA relative to the levels of total poly(A)⁺ RNA (Griffin and Morrison, 1985; Griffin et al., 1985; Griffin 1987, 1988) in PHF⁺ and PHF⁻ neurons in AD cortex.

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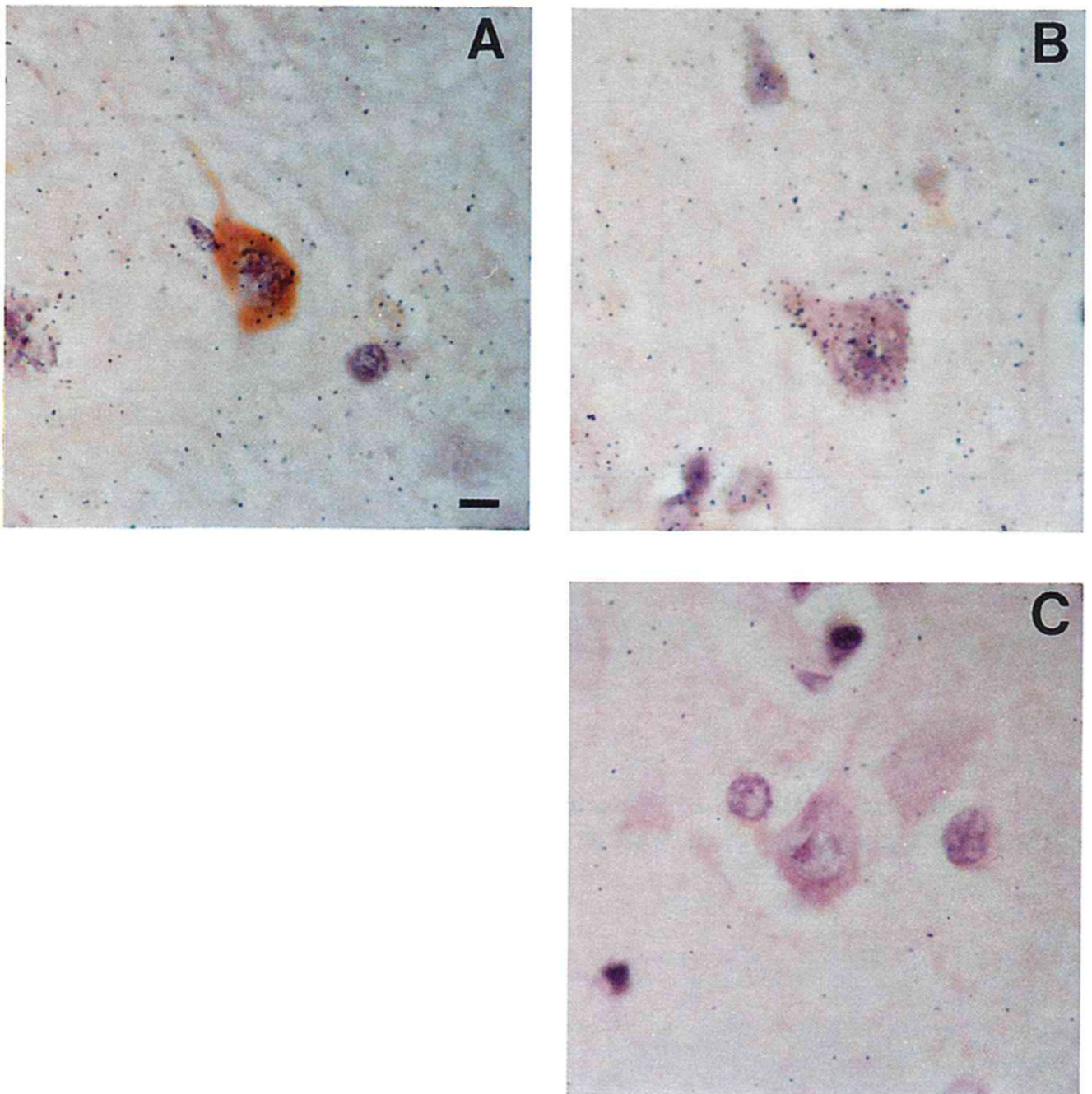


FIG. 1. Photomicrographs illustrating combined in situ hybridization and immunohistochemistry on 10 μm thick, formalin-fixed, paraffin-embedded sections of superior temporal gyrus from Alzheimer disease (AD) and age-matched control (AMC) brains. Photomicrographs of (A) a neuron with (brown–orange) and without (pink) PHF immunoreactivity in AD superior temporal cortex in situ hybridized with $[^3\text{H}]\text{poly}(\text{U})$; (B) PHF-negative (PHF $^-$) neuron in situ hybridized with $[^3\text{H}]\text{poly}(\text{U})$; and (C) PHF-negative neuron after pretreatment with ribonuclease A in low salt buffer. The bar (A) represents approximately 10 μm .

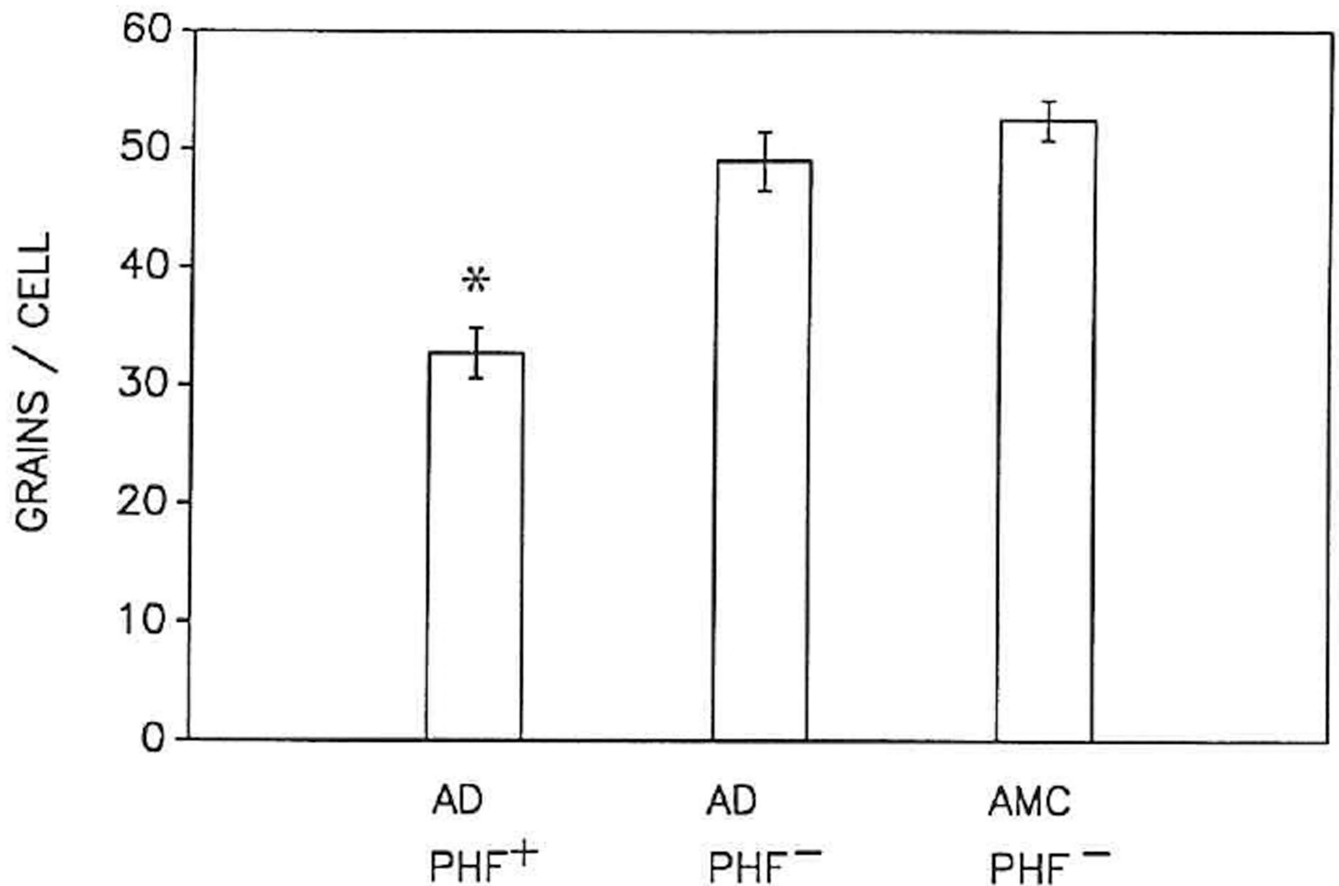


FIG. 2.

Bar graph representing the mean \pm SEM of autoradiographic silver grains above PHF-immunoreactive neurons (PHF⁺) and nonimmunoreactive (PHF⁻) neurons of similar cross-sectional area (approximately 300 μm^2) in tissue sections of superior temporal gyrus from Alzheimer disease (AD; $n = 4$) and age-matched control (AMC; $n = 4$) brains. Grains were counted above 20 PHF⁺ for comparison to 20 PHF⁻ neurons in the same AD section as well as 20 from AMC. The ranges in the number of grains were as follows: 17–49 per PHF⁺ cell in AD; 33–75 per PHF⁻ cell in AD; and 43–72 per PHF⁻ cell in AMC. The asterisk (*) denotes difference ($p < 0.05$) in the number of autoradiographic grains above PHF⁺ neurons compared to PHF⁻ neurons in either AD or AMC.

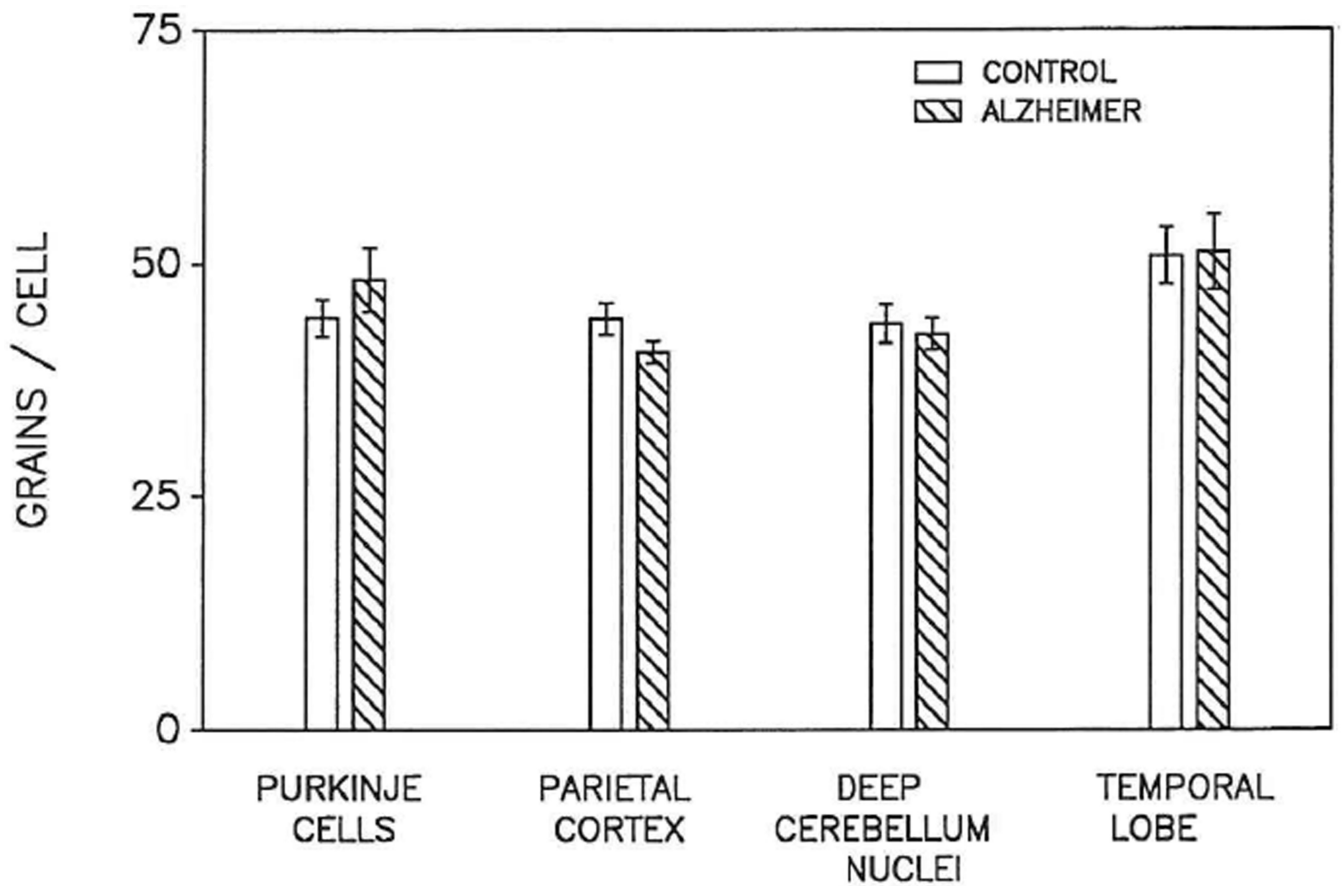


FIG. 3.

Bar graph representing the mean \pm SEM of autoradiographic silver grains above 20 neurons in tissue sections from postmortem parietal and temporal lobes and cerebellum from each of four patients who had Alzheimer disease (AD) for comparison to equal numbers of neurons of comparable size (approximately $300 \mu\text{m}^2$) in the same brain regions from four similar-aged individuals (AMC). The range of grains per cell was 39 to 104.