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BDNF and Full-length and Truncated TrkB Expression in Alzheimer Disease. Implications in Therapeutic Strategies

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Abstract. Brain-derived neurotrophic factor (BDNF), and full-length and truncated tyrosin kinase B receptor (TrkB) protein expression were examined by Western blotting and immunohistochemistry in the frontal cortex and hippocampus of individuals affected by long-lasting severe Alzheimer disease (AD) and age-matched controls. Since preliminary processing studies in the brains of rats have shown loss of immunoreactivity depending on the postmortem delay in tissue processing and on the type, duration, and temperature of the fixative solution, only human samples obtained up to 6 hours (h) after death for biochemical and morphological studies and fixed by immersion in 4% paraformaldehyde for 24 h for morphological studies were included in the present series. Decreased BDNF and full-length TrkB expression accompanied by increased truncated TrkB expression, as revealed by Western blotting, was observed in the frontal cortex of patients with AD. Immunohistochemistry disclosed reduced BDNF and full-length TrkB immunoreactivity in neurons. BDNF decrease was equally observed in tangle-bearing and non-tangle-bearing neurons, as revealed with double-labeling immunohistochemistry to BDNF and phosphorylated tau or phosphorylated neurofilament epitopes. Full-length TrkB immunoreactivity was largely decreased in tangle-bearing neurons, whereas only moderate decreases occurred in neurons with granulovacuolar degeneration. Strong BDNF immunoreactivity was observed in dystrophic neurites surrounding senile plaques, whereas strong TrkB expression occurred in reactive glial cells, including those surrounding senile plaques. Finally, truncated TrkB immunoreactivity was observed in individual neurons and in reactive glial cells in the cerebral cortex and white matter in AD. These results show decay in the expression of BDNF and TrkB in AD neurons, accompanied by altered BDNF and full-length and truncated TrkB expression in dystrophic neurites and reactive glial cells, respectively, in this disease. The present results demonstrate selective decline of the BDNF/TrkB neurotrophic signaling pathway in the frontal cortex and hippocampus in AD and provide supplemental data that may be relevant in discussing the suitability of the use of BDNF as a therapeutic agent in patients with AD.

Key Words: Alzheimer disease; BDNF; Full-length TrkB; Truncated TrkB.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors (1, 2), which binds specifically to the TrkB tyrosine kinase receptor, thus mediating neurotrophic signaling (3). TrkB is composed of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain which, once phosphorylated after neurotrophin binding, mediates intracellular signal transduction (3). In addition, the TrkB tyrosine kinase gene codes for truncated TrkB neurogenic receptors that lack the catalytic kinase domain (4, 5). Interestingly, truncated TrkB isoforms are mainly encountered in cultured glial cells, and in neuronal and non-neuronal cells in the normal adult central nervous system (6). BDNF promotes survival and growth of various nerve cell populations during normal development and following insults in the developing and adult brain (see 7 for review).

Several studies have shown modifications in the expression of BDNF and TrkB following acute insults to the nervous system. BDNF mRNA induction has been observed after experimental brain trauma (8, 9), whereas BDNF and TrkB mRNA expression is increased in spinal cord and facial neurons after axotomy (10, 11), and in the dentate gyrus following angular bundle transection (12). Transient forebrain ischemia in the rat is accompanied by increased BDNF and TrkB mRNA and protein expressions that correlate with regional differences in cellular resistance to ischemic damage and with the remodeling capacities of the injured areas (13–15). Induction of BDNF and TrkB mRNAs is also observed in the ipsilateral cortex outside the infarct and in the bilateral hippocampi following middle cerebral artery occlusion in the rat (16). Systemic administration of kainic acid at convulsant doses to the rat, which results in cell death in the entorhinal and piriform cortices, selected thalamic nuclei, and hippocampus, produces a complex time-dependent and cell-specific BDNF, and full-length and truncated TrkB responses (17–19). These data illustrate that BDNF and its specific receptors participate in the cellular responses to distinct experimental insults to the nervous system, which are currently used to simulate pathological situations in humans. Yet similar studies in real human

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neurodegenerative disorders, such as Alzheimer disease (AD), are scanty and difficult to perform for obvious reasons. This information in human cases is, nevertheless, critical not only to improve understanding about the possible involvement of neurotrophins in AD, but also to procure a baseline to rationalize the use of neurotrophins as possible therapeutic agents in specific human neurodegenerative diseases.

Previous studies have demonstrated reduced levels of BDNF mRNA, as shown with in situ hybridization and RNAase protection assay, in the hippocampus of individuals with AD (20, 21). Reduced BDNF protein levels have also been reported in the hippocampus and temporal cortex, as revealed with immunohistochemistry (22), and in the entorhinal cortex, as revealed with 2-site enzyme immunoassays (23) in patients with AD. Quantitative analysis of TrkB mRNA in situ hybridization signals in cells of the striatum and nucleus basalis of Meynert have failed to demonstrate significant differences between AD patients and control subjects (24). Yet immunohistochemistry has shown reduced expression of TrkB in the nucleus basalis (25), and abnormal TrkB expression in the temporal cortex and hippocampus, as well as the occurrence of full-length and truncated TrkB immunoreactivity in senile plaques in AD (26). Taken together, these findings indicate reduced BDNF expression and alterations in the distribution of the specific BDNF receptors in AD. However, it is important to elucidate whether there is any relationship between abnormal BDNF expression and neurofibrillary and neuritic pathology, and whether the abnormal TrkB expression occurs in neurons, glial cells, or dystrophic neurites in senile plaques.

Morphological and biochemical studies on human material obtained at postmortem are hampered by possible artifacts related to the postmortem delay between death and tissue processing (freezing or fixation of the tissue samples), and to the prolonged maintenance and inadequate temperature of the fixative solution. Minimizing pitfalls inherent to the suboptimal preservation of postmortem material is a requirement in the analysis of human neurodegenerative disorders. The present study first examines the conservation of BDNF, full-length, and truncated TrkB (respectively, TrkB and TrkB-TK) proteins in the brains of rats maintained under different protocols that mimic the procedures used in human cases, and, later, the expression of these proteins in AD brains and age-matched controls obtained at autopsy, analyzed under the very strict limitations derived from the experimental paradigms. Western blotting and simple and double-labeling immunohistochemistry have been used to learn about the expression levels, as well as the localization of BDNF, TrkB, and TrkB-TK in the frontal cortex and hippocampus of individuals with advanced AD.

MATERIAL AND METHODS

Experimental Procedures

Adult male Sprague-Dawley rats (200–300 g) from our own colony were killed under deep diethyl ether anesthesia, and the bodies were stored at 4°C for 0, 3, 6, 12, and 24 h. Later, the brains were removed from the skulls and processed for biochemical (n = 2 per each time-point) or morphological (n = 4 per each time-point) studies. For biochemical studies (gel electrophoresis and Western blotting), the cerebral cortex was dissected, frozen on dry ice and stored at −80°C until use. For morphological studies, the brains were fixed by immersion in 4% paraformaldehyde in phosphate buffered saline (PBS) for 48 h, washed in PBS, and immediately afterwards embedded in paraffin or cut with a vibratome. In addition, the brains of other rats maintained at 4°C for 6 h were fixed by immersion in 10% formalin for 7 or 15 days at room temperature (n = 4 per each time-point) and then embedded in paraffin or cut with a vibratome. Paraffin sections, 7-μm-thick, and vibratome sections, 30-μm-thick, were processed for immunohistochemistry. Animal welfare was conducted according to the regulations of the Real Decreto 223:1988 of the Spanish Government, which makes recommendations similar to those of the NIH report, *Public Health Service Policy on the Human Care and Use of Laboratory Animals.*

Human Cases

Samples of the frontal cortex (area 8) and hippocampus from 8 patients with AD (ranging from 71 to 82 years, mean age: 75.7 years; 3 men, 5 women), 6 age-matched controls with no neurological disease (ranging from 71 to 81 years, mean age: 74.8; 2 men, 4 women); and 2 patients with old cortical infarcts (aged 73 and 68 years; 2 men) were obtained at autopsy. The delay between death and tissue processing was between 2 and 6 h. All AD cases had suffered from long-lasting severe sporadic AD (mean duration 7 years), and all of them were categorized as stage VI of Braak and Braak (27) on neuropathological examination. None of the control cases had neurofibrillary tangles in the frontal cortex, but there were a few neurofibrillary tangles (3 neurons per section) in the entorhinal cortex in one of them. Diffuse plaques were seldom observed in the neocortex and hippocampus in control cases. For morphological studies, the samples were fixed with 4% paraformaldehyde in PBS for 48 h and embedded in paraffin or cut with a vibratome. The neuropathological examination was carried out in every case (AD cases and controls). For this purpose dewaxed paraffin sections, 7-μm-thick, were stained with hematoxylin and eosin, luxol fast blue-Klüver Barrera, and methenamine silver (PAM), or processed for immunohistochemistry following the avidin-biotin-peroxidase method (ABC, Vector, Vectastain) to phosphorylated neurofilaments of 200 kD (clone RT97, Boehringer-Mannheim; dilution 1:50), phosphorylated tau (Sigma; dilution 1:400), βA4-amyloid (Boehringer-Mannheim; dilution 1:5), glial fibrillary acidic protein (GFAP, Dako, Dakopats; dilution 1:250) and ubiquitin (Dako; dilution 1:200). For gel electrophoresis and Western blotting, fresh samples of the frontal cortex from patients with AD and age-matched controls were rapidly frozen on dry ice and stored at −80°C until use. BDNF, and

full-length and truncated TrkB immunohistochemistry were carried out in all the control and pathological cases. Samples for Western blotting of the frontal cortex were available from 4 control and 4 AD cases, whereas samples of the hippocampus were obtained from 4 control and 6 AD cases.

Gel Electrophoresis and Western Blotting

The samples were homogenized in buffer containing 100 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium decolate, 0.1% SDS, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, leupeptin, and pepstatin (Sigma). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using a mini-protean system (Bio-Rad) with molecular weight standards (Bio-Rad). Equal amounts of protein (20 μg) were loaded in each lane with loading buffer containing 0.125 mM Tris (pH 6.8), 20% glycerol, 10% mercaptoethanol, 4% SDS, and 0.002% bromophenol blue. Samples were heated at 95°C for 10 min before gel loading. Protein analyzed on gel electrophoresis was transferred to nitrocellulose membranes (Amersham) using an electrophoretic transfer system (Trans-blot semi-dry Transfer Cell, Bio-Rad) at 40 mA for 1 h. After that, the membranes were blocked with T-TBS containing 5% skimmed milk, 2% BSA (Sigma), and 1% normal serum (Vector). Following this, the membranes were incubated at 4°C overnight with one of the primary antibodies. The BDNF rabbit polyclonal antibody (sc 546, Santa Cruz Biotechnology) is raised against a peptide corresponding to amino acids 128–147 mapping at the carboxy terminus of BDNF of human origin, which is identical to the corresponding mouse sequence. The full-length TrkB rabbit polyclonal antibody (sc 12, Santa Cruz Biotechnology) is raised against a peptide corresponding to amino acids 794–808 mapping adjacent to the carboxy terminus of the precursor TrkB gp 145 of mouse origin. The truncated rabbit polyclonal antibody TrkB-TK (sc 119, Santa Cruz Biotechnology) is raised against an epitope corresponding to amino acids mapping at the carboxy terminus of the precursor form of the truncated TrkB protein gp 95 of mouse origin, identical to the corresponding rat sequence. The specificity of these antibodies was tested by preincubating the antibodies with the antigenic peptides (sc 546p, sc 12p and sc 119p, also available from Santa Cruz Biotechnology). After washing, the membranes were incubated with anti-rabbit IgG labeled with HRP (Dako) diluted 1:1,000 for 1 h at room temperature. The membranes were washed again and developed with the chemiluminescence ECL Western blotting system (Amersham) followed by apposition of the membranes with autoradiographic films (Hyperfilm ECL, Amersham). Control of protein content in each lane was evaluated by the staining of selected gels with Coomassie blue and the membranes with Ponceau (Sigma). Western blots recognized specific bands at the appropriate molecular weights of BDNF, TrkB, and TrkB-TK. Quantification of the density of the bands was carried out as follows. The bands were scanned with the Epson GT-8000 scanner at a resolution of 400 dpi. The density of the signal was analyzed with the IMAT program, and the results were expressed for each protein as the percentage of decrease in the intensity of the signal referred to the controls.

BDNF and Full-length and Truncated TrkB Immunohistochemistry

Tissue sections were incubated with 0.3% hydrogen peroxide and methanol for 20 min, and with normal serum for 30 min at room temperature. Immediately afterwards, the sections were incubated at 4°C overnight with the same primary antibodies to BDNF, TrkB, and TrkB-TK utilized for Western blotting. The antibodies were used at dilutions of 1:500 in vibratome sections and 1:200 in paraffin sections. Then, the sections were incubated with anti-rabbit biotinylated anti-IgG (Vector) at a dilution of 1:200 and with ABC at a dilution of 1:100 for 1 h, each step at room temperature. The peroxidase reaction was visualized with 0.05% diaminobenzidine and 0.01% hydrogen peroxide. Paraffin sections were lightly counterstained with hematoxylin. The specificity of the immunoreaction was also tested in tissue sections by preincubating the BDNF, TrkB, and TrkB-TK antibodies with the corresponding antigenic peptides (sc 546p, sc 12p, and sc 119p) prior to immunohistochemistry. No immunostaining was observed in these sections. Tissue samples from control and pathological cases were processed in parallel to avoid day-to-day variations in the immunostaining.

Double-labeling immunohistochemistry was conducted by incubating the sections following a 2-step protocol. Briefly, the sections were first incubated with one of the primary antibodies, and the immunoreaction was visualized with diaminobenzidine and hydrogen peroxide as before. Subsequently, the sections were incubated with the second primary antibody, and the immunoreaction was visualized with 0.01% benzidine hydrochloride, 0.025% sodium nitroferricyanide in 0.001% M sodium phosphate buffer (pH 6.0), and 0.005% hydrogen peroxide. The first primary antibody was recognized by a brown homogeneous precipitate, whereas the second primary antibody was recognized by a dark blue granular precipitate.

RESULTS

Effects of Postmortem Delay and Tissue Processing in BDNF and Full-length and Truncated TrkB Expression

A decay of 15% in BDNF immunoreactivity, as revealed with Western blotting, was observed in the brains of rats maintained at 4°C for 24 h previous to their freezing and storage at −80°C. A reduction of 20% and 33% in full-length and truncated TrkB immunoreactivity was encountered in samples processed at 12 h, whereas a reduction of 37% and 60%, respectively for full-length and truncated TrkB, occurred at 24 h (Fig. 1). A similar decrease in the expression with time was found in tissue sections of rat brains fixed in toto by immersion in 4% paraformaldehyde for 48 h at 4°C and then processed for immunohistochemistry. Preserved BDNF and full-length and truncated TrkB immunoreactivity was observed up to 6 h. However, a dramatic reduction in BDNF and full-length and truncated TrkB immunoreactivity occurred in the brains of rats maintained at 4°C for 6 h and then fixed by immersion with 10% formalin for 7 days or 15 days at room temperature (Fig. 2). These results have limited the examination of human material to a maximum of 6

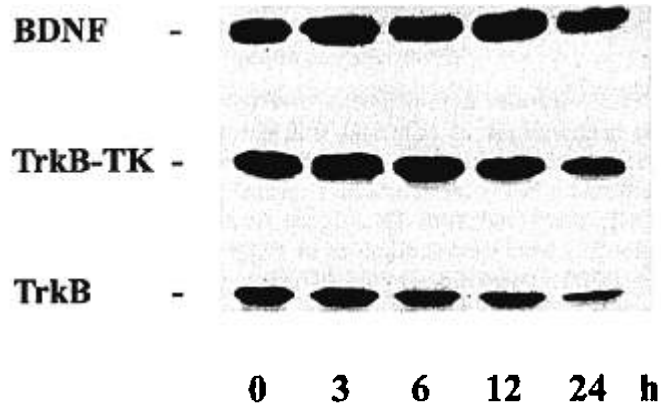

Fig. 1. Western blots to BDNF and truncated and full-length TrkB (TrkB-TK and TrkB, respectively) from cerebral cortex homogenates of rats maintained at 4°C for 0, 3, 6, 12, and 24 h after death. The brains were then frozen at −80°C and processed for gel electrophoresis. Reduced BDNF immunoreactive expression occurs in samples with postmortem delays of 24 h, whereas reduced TrkB-TK and TrkB expression is already observed at 12 h.

h of postmortem delay for biochemical and morphological studies, and to the fixation of the tissue samples for 48 h in 4% paraformaldehyde at 4°C for immunohistochemistry.

BDNF and Full-length and Truncated TrkB in Human Frontal Cortex and Hippocampus

BDNF immunoreactivity was observed in the majority if not all neurons of the frontal cortex (Fig. 3A). Similarly, BDNF immunoreactivity was found in neurons of the pyramidal cell layer (Fig. 3C) and in neurons of the plexiform layers of the hippocampus, as well as in granule neurons of the dentate gyrus and polymorphic neurons of the hilus (Fig. 3D). BDNF immunoreactivity was also observed in reactive astrocytes in the samples corresponding to the patients with old cerebral infarcts (Fig. 3E). Preincubation of the BDNF antibody with the antigenic peptide prior to immunohistochemistry resulted in the blockade of the immunoreaction (Fig. 3B). TrkB was expressed in the majority of cortical and hippocampal neurons (Fig. 3F). In addition, full-length TrkB was found in glial cells in the gray matter (Fig. 3G) and subcortical white matter (Fig. 3H). Finally, the cerebral cortex was diffusely immunostained with the anti-TrkB-TK antibody, although truncated TrkB-TK immunoreactivity was mainly observed in astrocytes of the subcortical white matter (Fig. 3I). These results are in agreement with previous studies on the expression of BDNF and their receptors in neurons of the cerebral cortex and hippocampus (28), and with the expression of BDNF and full-length and truncated TrkB in glial cells under appropriate conditions (6, 29–31).

BDNF and Full-length and Truncated TrkB in AD

Western blots of the frontal cortex demonstrated a decrease of BDNF ranging from 11% to 42% (42%, 11%, 15%, and 24% for each case; mean 23%) in individuals with AD when referred to age-matched control levels. Marked decrease of full-length TrkB, ranging from 24% to 71% (71%, 24%, 48%, and 69% for each case; mean 53%), was also observed in AD. Interestingly, truncated TrkB expression was increased in those individuals in whom the decrease of BDNF and full-length TrkB was particularly severe (Fig. 4).

BDNF immunoreactivity was decreased in cortical and hippocampal neurons in patients with AD (Fig. 5A, B). Tangle-bearing and non-tangle-bearing neurons revealed with double-labeling immunohistochemistry to BDNF and either phosphorylated tau or phosphorylated neurofilament epitopes were equally affected (Fig. 5C). Strong BDNF immunoreactivity was, however, observed in dystrophic neurites surrounding amyloid deposition in senile plaques (Fig. 5D, E), whereas no BDNF immunoreactivity was observed in reactive glial cells in AD. Reduced full-length TrkB immunoreactivity also occurred in neurons of the cerebral cortex and hippocampus in individuals with AD. Reduction was severe in neurons with neurofibrillary degeneration in the cerebral cortex and hippocampus, but moderate in hippocampal neurons with granulovacuolar degeneration (Fig. 6A–E). Strong TrkB immunoreactivity occurred in scattered glial cells in the neuropil and in reactive glial cell clusters surrounding senile plaques (Fig. 6F). Finally, truncated TrkB immunoreactivity, not related to tangle formation, was observed in scattered neurons of the cerebral cortex, as well as in glial cells in the cerebral cortex and subcortical white matter (Fig. 6G–I). Dystrophic neurites were not stained with anti-TrkB or anti-TrkB-TK antibodies.

DISCUSSION

Delayed tissue processing results in a decay of BDNF and full-length and truncated TrkB immunoreactivity, with particularly ravaging consequences in postmortem material preserved in formalin for several days, as shown in the present study. For this reason, rigorous limitations conditioned the selection of cases in the present series.

The present results confirm BDNF decrease in the cerebral cortex and hippocampus of individuals with AD (20–23). In addition, they show that BDNF decrease is not only the consequence of nerve cell loss but also, more importantly, the consequence of reduced BDNF expression in individual neurons. Furthermore, reduced BDNF expression is not associated with neurofibrillary tangle formation, as BDNF is equally found in tangle-bearing and non-tangle-bearing neurons. Yet BDNF is strongly expressed in dystrophic neurites of senile plaques, as revealed with double-labeling immunohistochemistry to

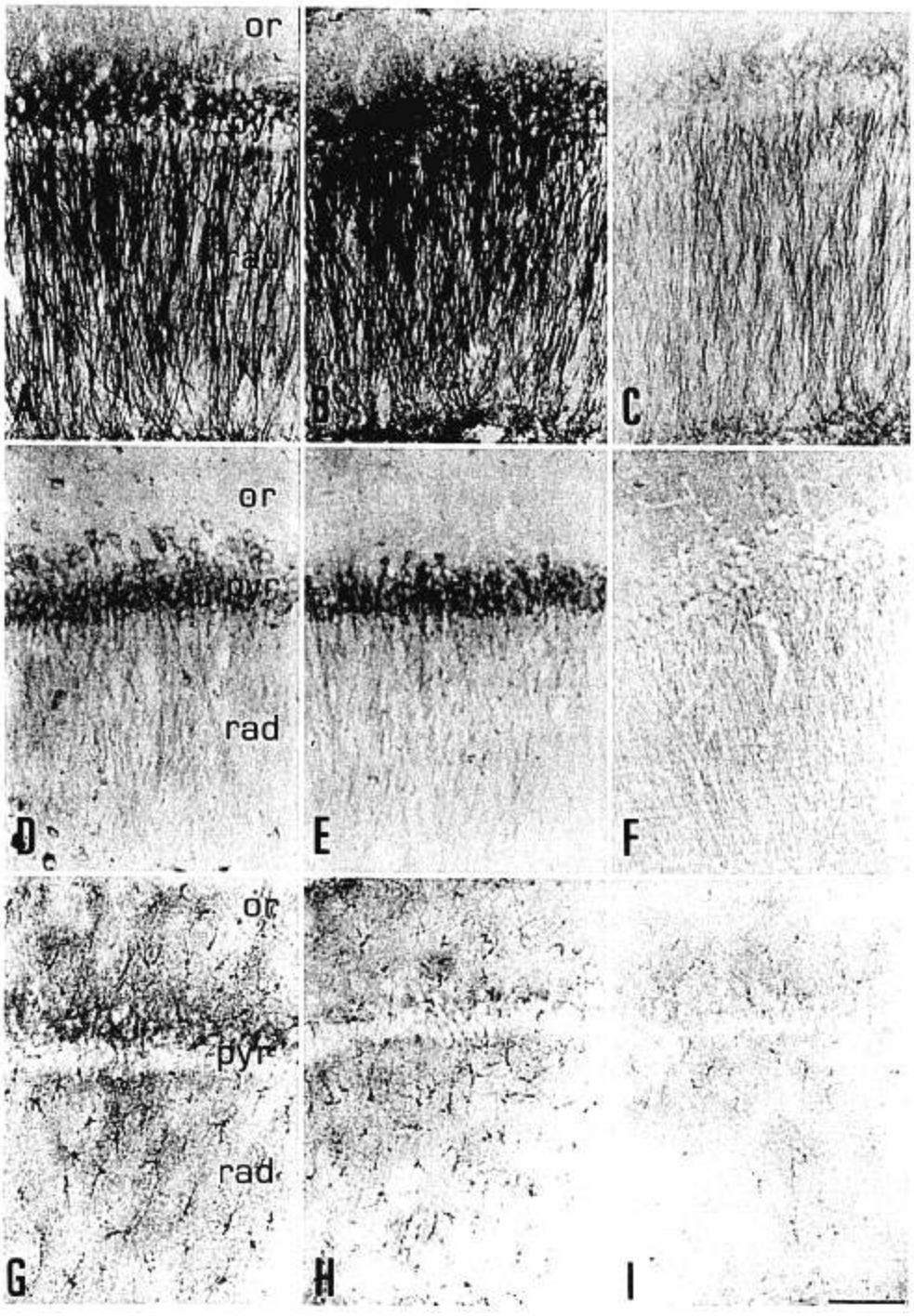

Fig. 2. BDNF (A–C), full-length TrkB (D–F) and truncated TrkB (G–I) immunoreactivity in the hippocampus of rats, the brains of which were immediately fixed after death by immersion in 4% paraformaldehyde at 4°C for 48 h (A, D, G); or stored at 4°C for 6 h and then fixed by immersion in 4% paraformaldehyde at 4°C for 48 h (B, E, H); or stored at 4°C and then fixed by immersion in 10% formalin at room temperature for 7 days (C, F, I). Marked reduction in BDNF, full-length and truncated TrkB immunoreactivity is observed in samples fixed by immersion in formalin for 7 days. Vibratome sections processed free-floating without counterstaining. Key: or = stratum oriens; pyr = stratum pyramidale; rad = stratum radiatum of the CA1 area. Bar = 50 μm.

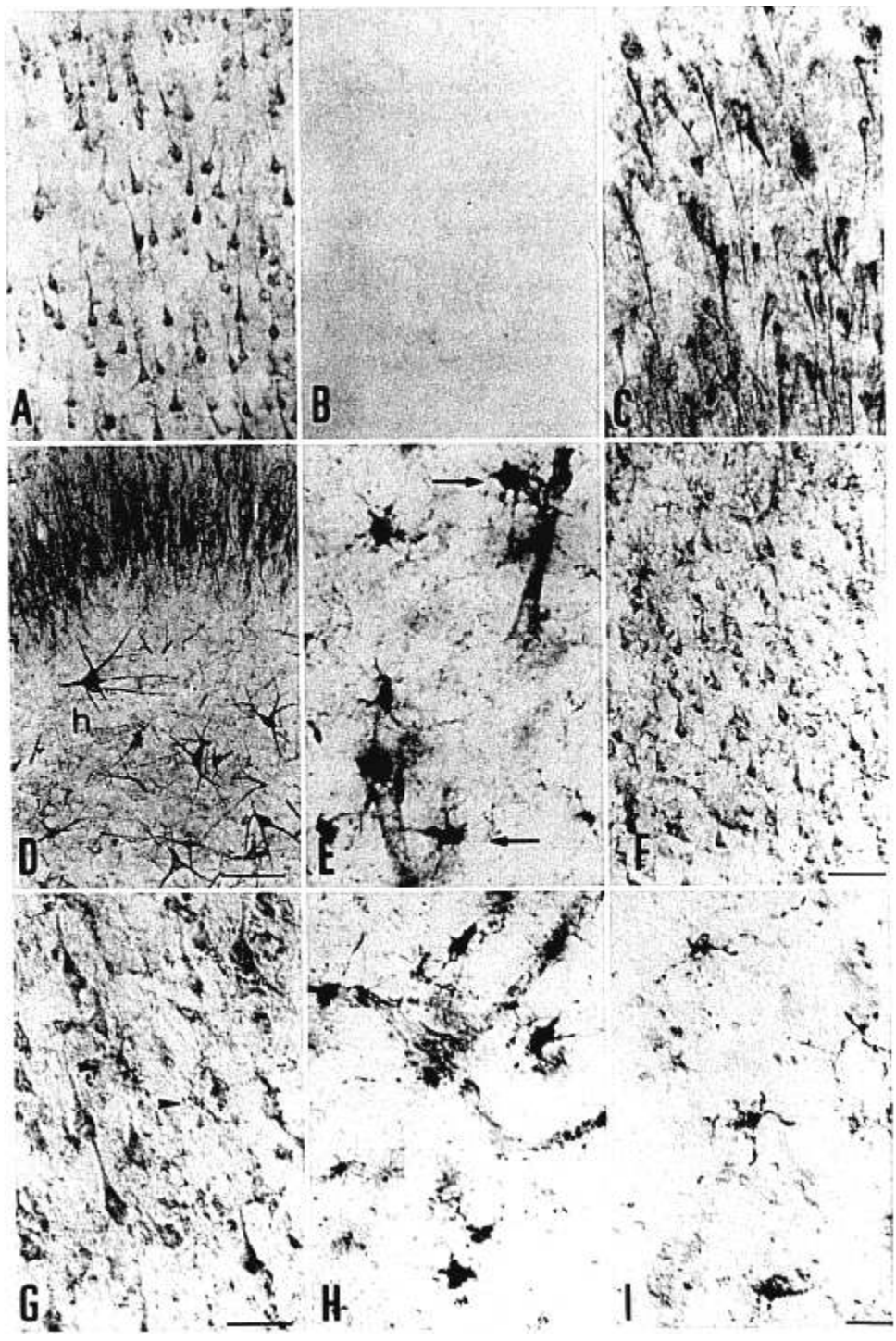

Fig. 3. BDNF (A–E), full-length TrkB (F–H) and truncated TrkB (I) immunoreactivity in the frontal cortex (A, B, F, G), CA1 area (C), dentate gyrus and hilus (D) of the hippocampus, subcortical white matter (E, H, I) in the normal adult human brain (A–D, F–I), and in one patient with old cerebral infarcts (E). BDNF immunoreactivity is observed in neurons of the cerebral cortex (A). The immunoreaction is abolished by preincubating the BDNF antibody with the antigenic peptide prior to immunohistochemistry (B). BDNF immunoreactivity is also observed in neurons of the CA1 area of the hippocampus (C) and in granule cells (gr) and hilar neurons (h) of the dentate gyrus (D). Although normal astrocytes are not stained with anti-BDNF antibodies, reactive astrocytes in the vicinity of old infarcts show strong BDNF immunoreactivity (E); stained cells with podocytes embracing small blood vessels are marked with arrows. TrkB immunoreactivity is found in cortical neurons (F, G), as well as in glial cells in both cerebral cortex (arrowheads in G) and subcortical white matter (H). Finally, truncated TrkB immunoreactivity is observed in glial cells in the subcortical white matter (I). Vibratome sections processed free-floating without counterstaining. A, B, C, F, bar in F = 50 μm; E, H, I, bar in I = 10 μm; G, bar = 25 μm; D, bar = 100 μm.

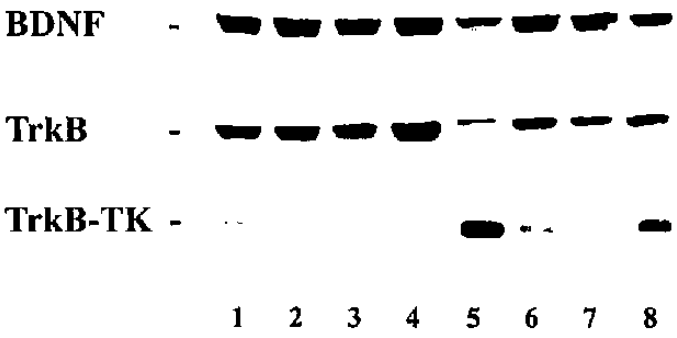

Fig. 4. Western blots to BDNF, and full-length and truncated TrkB (TrkB and TrkB-TK, respectively) from frontal cortex homogenates of AD cases (lanes 5–8) and age-matched controls (lanes 1–4). Reduction in BDNF and TrkB expression is observed in individuals with AD whereas increased TrkB-TK expression is particularly evident in those cases with lower levels of BDNF and TrkB.

BDNF and either phosphorylated tau or phosphorylated neurofilament epitopes, here used as markers of dystrophic neuronal processes. Altered levels and splicing of the amyloid precursor protein (APP) are not accompanied by changes in BDNF transcripts in the adult rat hippocampus (32), thus suggesting that accumulation of BDNF in dystrophic neurites is not the consequence of abnormal APP processing. Alternatively, strong BDNF immunoreactivity in dystrophic neurites could be the result of impaired BDNF transport and release. It is not clear whether BDNF accumulation in dystrophic neurites may have any functional effect.

The present results have also shown that full-length TrkB receptor expression is reduced in neurons of the cerebral cortex and hippocampus, thus indicating not only reduced BDNF expression but also possible decreased BDNF signaling in AD brains. Neurons with neurofibrillary degeneration are particularly prone to low TrkB expression. In contrast, full-length and truncated TrkB are expressed in reactive glial cells, and truncated TrkB in individual neurons, thus probably accounting for increased truncated TrkB levels in patients with AD. The functions of truncated TrkB isoforms are not known although various roles have been proposed, including inhibition signaling of the full-length receptor, restriction of the diffusion of neurotrophin from the sites of release, and positive regulation of BDNF action by presenting BDNF to full-length receptors (6, 33). Previous studies have demonstrated that truncated TrkB receptors restrict the availability of BDNF during development (34, 35), and that truncated TrkB on non-neuronal cells inhibits BDNF-induced outgrowth in vitro (36). Whether reactive astrocytes in AD participate in the limitation of the plastic capacities of neurons by impeding the access of BDNF to their positive targets remains unknown. Finally, although TrkB is expressed in non-neuronal cells surrounding senile plaques, we have been unable to confirm TrkB expression in the amyloid core of senile plaques (26). Postmortem delays of up to 34 h and predominance of positive plaques in cases with the longest postmortem delays in the later study (26), together with differences in the methods employed, may explain these discrepancies.

These data on BDNF, full-length TrkB, and truncated TrkB expression differ from those reported for Nerve Growth Factor (NGF) and both high-affinity (TrkA) and low-affinity (p75) NFG receptors in AD. Preserved or increased NGF mRNA and protein is observed in the neocortex and hippocampus (23, 37–41), whereas NGF expression is decreased in the nucleus basalis (41). TrkA expression is decreased in various regions, including the nucleus basalis (25, 42, 43), frontal and parietal cortices (43, 44), and molecular layer of the dentate gyrus (26), although it is preserved in the striatum (42). In spite of the total TrkA decrease, it is worth pointing out that strong TrkA expression occurs in reactive astrocytes in AD (26, 45). Finally, p75 expression, although stable in the nucleus basalis (46), is decreased in the fimbria and alveus in AD when compared with age-matched controls (47). Considering all this information, it is clear that the NGF and BDNF neurotrophic signaling pathways are differentially altered in Alzheimer disease.

Interest has emerged on the use of neurotrophins in new treatment strategies for human neurodegenerative diseases. The generalized decrement of BDNF in AD is an argument to support therapeutic strategies based on the administration of BDNF in this disease. In this line, it has been demonstrated that BDNF administration protects basal forebrain cholinergic neurons from degenerative changes after axotomy in the adult rat (48, 49), and prevents hippocampal cell damage following transient forebrain ischemia in adult rats and gerbils (50–52). In addition, it has been shown that BDNF modulates synaptic innervation density, enhances synaptic transmission, and upregulates the expression of proteins implicated in the exocytosis of synaptic vesicles (53). In spite of these positive effects, it must be stressed that BDNF may exacerbate excitotoxic neuronal injury to cultured cortical neurons (54) and kainic acid-induced neuronal damage in the hippocampus (55). Furthermore, high doses of BDNF downregulate TrkB mRNA and TrkB protein in hippocampal cultures (56), reduce TrkB mRNA and TrkB protein, and tyrosine phosphorylation in cultured cortical neurons (57), and binding to cell-surface receptors in cultured cells (58). Yet low doses of BDNF upregulate TrkB protein and prevent the death of CA1 neurons following transient forebrain ischemia (52). Whether exogenous administration of BDNF at appropriate doses could normalize full-length TrkB protein expression in AD, thus providing target neurons the adequate levels of ligand to BDNF surplus, is a crucial aspect in the treatment of the disease.

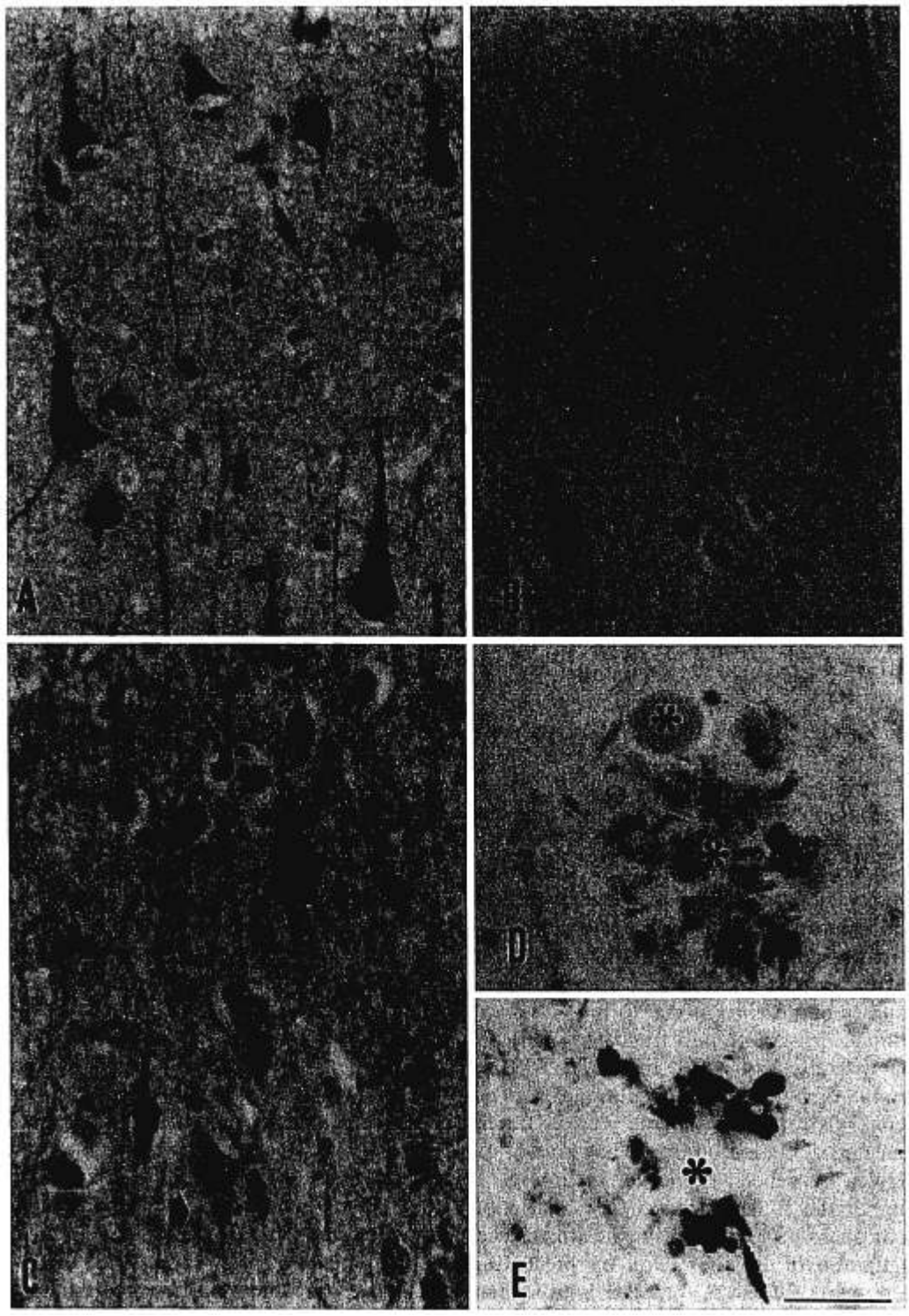

Fig. 5. BDNF immunoreactivity in the frontal cortex in control (A) and AD (B–E) cases. Reduced BDNF immunoreactivity is found in AD neurons (A, B). Immunoreactivity is, however, equally present in tangle-bearing (arrows) and non-tangle-bearing neurons (C). Strong BDNF immunoreactivity is observed in dystrophic neurites surrounding senile plaques, as seen with simple (D) and double-labeling (E) immunohistochemistry. Paraffin sections slightly counterstained with hematoxylin. C, E: double-labeling immunohistochemistry to BDNF and phosphorylated neurofilament epitopes (clone RT97). Asterisks = amyloid deposition. Bar = 25 μm.

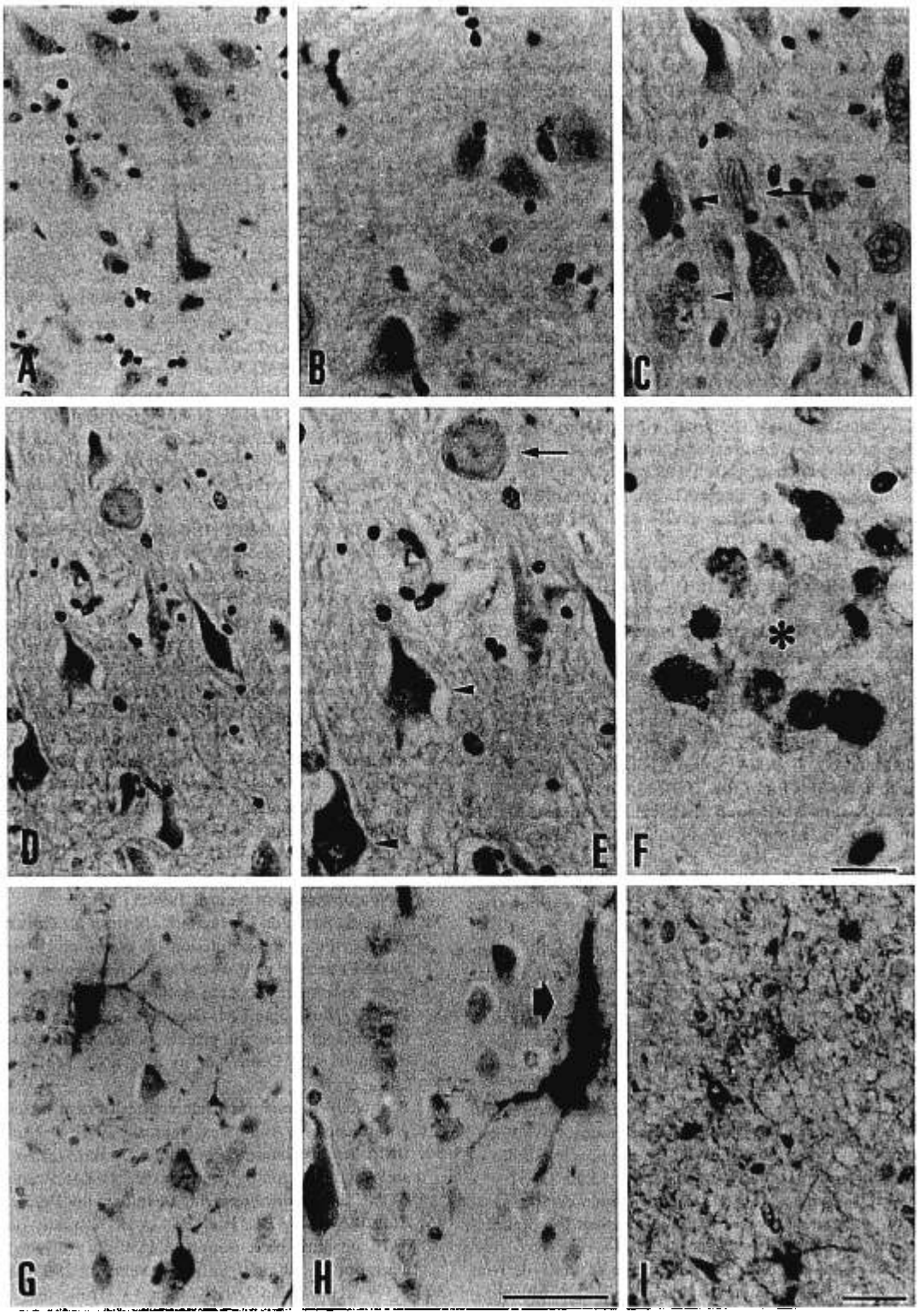

Fig. 6. Full-length TrkB (A–F) and truncated TrkB (G–I) immunoreactivity in the hippocampus (A–F) and cerebral cortex (G–I) in control (A, B) and AD (C–I). Reduced TrkB immunoreactivity is found in individuals with AD (A–E). Immunoreactivity is markedly decreased in association with neurofibrillary tangles (arrows in C and D), whereas immunoreactivity is moderately reduced in neurons with granulovacuolar degeneration (arrowheads in C and E). Strong TrkB expression is found in glial cells surrounding senile plaques (F). Truncated TrkB immunoreactivity is observed in scattered neurons in the cerebral cortex (G, H), some of them with tortuous profiles (arrow in H), as well as in reactive astrocytes in the subcortical white matter (I). Paraffin sections slightly counterstained with hematoxylin. Asterisk = amyloid deposition. A, D, G, I, bar in I = 25 μm; B, C, E, H, bar in H = 25 μm; F, bar = 10 μm.

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