Acta Neuropathol (1992) 83: 170 - 178

Acta Neuropathologica

A comparative study of histological and immunohistochemical methods for neurofibrillary tangles and senile plaques in Alzheimer's disease*

P. G. Vallet[1], R. Guntern[1], P. R. Hof[2,3], J. Golaz[1], A. Delacourte[5], N. K. Robakis[2,4], and C. Bouras

[1] Department of Psychiatry, University of Geneva, 2 ch. du Petit Bel-Air, CH-1225 Chêne-Bourg (Geneva), Switzerland
[2] Fishberg Research Center for Neurobiology, and Departments of [3] Geriatrics and Adult Development, and [4] Psychiatry Mount Sinai School of Medicine, New York, NY 10029, USA
[5] INSERM U156, Laboratoire de Neurosciences, Place de Verdun, F-59045 Lille, France

Received October 1, 1990/Revised March 8, 1991/Revised, accepted September 27, 1991

Summary. Several studies have demonstrated that the accurate visualization and quantification of pathological lesions in neurodegenerative disorders depend on the reliability of staining methods. In an attempt to gain a better assessment of the density and distribution of the neuropathological markers of Alzheimer's disease, we compared the staining efficiency of a modified thioflavine S protocol for neurofibrillary tangles (NFT) and senile plaques (SP) to different argentic impregnation techniques (Bielchowsky, Gallyas, Globus, Campbell-Switzer-Martin) and to immunohistochemical stainings obtained with two different antibodies against the amyloid β protein A4 and the microtubule-associated tau protein. The modified thioflavine S technique (MTST) detects up to 60% more SP and up to 50% more NFT than the Bielschowsky and Globus methods, respectively. The results obtained with the specific antibodies are comparable to those obtained with the MTST, but these immunotechniques are more expensive and time consuming for routine neuropathological evaluation, and the appropriate antibodies are not always commercially available. Furthermore, the morphological appearance of NFT and SP with MTST is greatly improved when compared to the classical thioflavine S and the increased signal-to-noise ratio between specifically stained structures and background permits an accurate semi-automatic quantification.

Key words: Senile plaques – Neurofibrillary tangles – Alzheimer's disease – Thioflavine S – Amyloid β-protein

Clinical criteria are used to establish a diagnosis of probable Alzheimer's disease (AD) [35], but a definite diagnosis requires the histological demonstration of senile plaques (SP) and neurofibrillary tangles (NFT) [20, 31]. Both the presence and the density of these neuropathological markers constitute crucial criteria for the characterization of AD [19, 36, 42]. In addition, the variations in the regional and laminar distribution patterns of SP and NFT [1, 34, 37, 39, 40] may lead to the isolation of AD subtypes with specific neurological symptoms [3, 15, 21, 23, 25, 29].

Routine staining methods currently used include the modified Bielschowsky and the thioflavine S techniques [4, 36, 46]. However, many other methods are available such as Campbell-Switzer-Martin [6], Gallyas [12], Globus [14] and Bodian techniques. The Bodian method is the less sensitive method [33, 44–46] and was not used in the present study.

Several immunohistochemical methods are used together with the "classical" techniques to demonstrate amyloid or preamyloid deposition, paired helical filaments and several abnormally phosphorylated proteins [30, 43]. In the present study we have used two highly specific antibodies, one to the amyloid β protein A4 [32, 38] and the other to the microtubule-associated protein tau [7, 8], to compare the immunohistochemical staining of senile plaques and NFT to the staining obtained with the MTST or classical methods.

Our results demonstrate that the MTST [18] is the easiest to perform and the less expensive, displays a much higher sensitivity than argentic and classical thioflavine S methods and is extremely reliable for routine diagnostic evaluation as well as for detailed quantitative neuropathological investigations.

Materials and methods

Seven brains from patients with clinically diagnosed and neuropathologically confirmed Alzheimer's disease (80.5 ± 10.5 years old) were used in the present study. Tissues were obtained at autopsy (post-mortem delay up to 36 h; mean 14.5 h, fixed by immersion in 15% (v/v) formalin for 30 days and then stored in 5% formalin for several years. Hippocampal and parahippocampal regions at the level of the mid part of the hippocampus were systematically

* Supported in part by the Brookdale Foundation and the American Health Assistance Foundation (to P.R.H.) and the NIH (AG05138 and AG08200 to N.K.R.)

Offprint requests to: P. G. Vallet (address see above)

sampled from each brain. Frontal (Brodmann areas 9, 10, 11, 12) and temporal cortices (Brodmann areas 20, 21, 28, 36) were removed only from one brain. For each block, a series of serial frozen sections (30 μm thick) were made with a Jung-freezing microtome (CO_2) and immersed in H_2O.

The 1st, 3rd, 5th, 7th, 9th and 11th sections were stained with MTST [16, 18]. Thioflavine S method [41] was applied to the 10th and 12th, Globus method [14] to the 2nd, 4th, 13th, 15th, 17th and 19th, Gallyas method [12] to the 6th, 8th, 14th, 16th and modified Bielschowsky [46] to the 18th and 20th. In addition, similar series including Bielschowsky versus MTST, Campbell-Switzer-Martin staining [6] versus MTST, anti-amyloid β protein A4 versus MTST and anti-tau versus MTST on adjacent sections were realized.

The histochemical procedure for the MTST was performed as previously described [18]. Floating sections were treated with 0.25 % $KMnO_4$ for 20 min and bleached in a solution consisting of 1 % $K_2S_2O_5$ and 1 % oxalic acid for 2 min. Afterwards the sections were treated with a solution containing 1 g NaOH and 3 ml of 30 % H_2O_2 in 100 ml H_2O for 20 min and dipped for 5 s in 0.25 % acetic acid. Between each step, sections were washed in H_2O. The sections were then mounted on albumin-coated slides, dried, rehydrated and subsequently stained with thioflavine S diluted up to 0.0125 % in 50 % ethanol for 3–5 min. Finally, sections were rinsed twice in 50 % ethanol and twice in H_2O and coverslipped with glycerin-H_2O (3:1). Deparaffinized floating sections (30 μm) can also be stained with MTST using the same protocol. For comparison, adjacent sections were stained using the "classical" thioflavine S method as follows: floating sections were stained for 5 min in a 1 % thioflavine S aqueous solution and were differentiated in 70 % alcohol (3–5 min). After washing in H_2O, sections were mounted and coverslipped with glycerin-H_2O (3:1) [41].

The Globus stain [14], used for routine diagnosis in our laboratory, was modified as follows: frozen sections were immersed in 5 % bromhydric acid – 5 % formalin neutralized by 2 % $CaCO_3$ for 2 h, rinsed twice in H_2O and dipped in 1 % Ag_2CO_3 solution containing 4 % pyridine (Merck) at 37 °C for 45 min. Sections were then rinsed again and placed in 20 % neutral formalin for 45 min. They were mounted on albumin-coated slides and dried. After rehydration, sections were dipped in 1 % $HAuCl_4$ solution until a grey tint was obtained. Subsequently they were placed in 5 % $Na_2S_2O_4$ for 20 min to stabilize the staining and finally washed in H_2O, dehydrated, and mounted in Entellan (A. Schoeb, personal communication).

The Bielschowsky staining method was performed according to the Yamamoto and Hirano modification using 20 % silver nitrate [46], the Gallyas staining following the original description [12] and the Campbell-Switzer-Martin method from Campbell et al. [6].

Immunohistochemistry with anti-β protein A4 and anti-tau was performed according to Guntern et al. [17]. Highly specific and previously characterized antibodies to the amyloid β protein A4 [32, 38] and the tau protein [7, 8, 11] were incubated at 1:4000 overnight after pretreatment of the tissues [17]. Following incubation the section were processed by the PAP method with 3,3′-diaminobenzidine used as chromogene.

On each section, pathological lesions were counted using an ocular reticule at a magnification of X 100 (field: 1 mm^2). Ten selected fields [CA1, CA3 and subiculum) containing lesions were surveyed and compared to the same field on adjacent sections. The same protocol was used to count SP and NFT in other cortical areas. Thioflavine S- and MTST-stained sections were analyzed under a Zeiss Axiophot fluorescence light microscope (BP 485 nm).

The high signal-to-noise ratio between labeling and background obtained with MTST, Gallyas and immunostaining is highly suitable for the semi-automatic quantification of the lesions using a computer-assisted microscopy system consisting of a Zeiss Axioplan microscope, a high sensitivity LH-4036 camera (LHESA Electronic), a COMPAQ Deskpro 386/20 microcomputer and a SAMBA™ 2005 software system developed by TITN Inc. (ALCATEL, Grenoble, France). To count semi-automatically SP and/or NFT stained with MTST in a predefined area, all the stained profiles are selected by thresholding, the results being a series of binary mask representations. For each surveyed field, labeled isolated points and artifacts, determined by size and by form, can be automatically removed. Moreover, the operator can clear any irrelevant element by manually editing the mask.

The difference between two different methods in densities of stained lesions in each field were compared on adjacent sections using a two-sided paired Student's *t*-test. Finally the time consumption for performing each method and costs were also assessed and compared.

Results

Among the five histological staining methods, we found that the MTST gave the best quantitative and qualitative results for the detection of SP and NFT (Figs. 1–3). The lesion densities assessed with the different histological procedures are presented in Table 1. The most striking differences in lesion counts were observed between MTST and the Bielschowsky method (59 % more SP with MTST in hippocampus; Fig. 2C, D). The Globus method stained only 54 % of SP and 52 % of NFT observed with MTST in the hippocampus (Figs. 1C, D; 2A, B). In the other frontal and temporal areas surveyed, 71 % and 76 % of SP were stained with argentic impregnation as compared to MTST. The difference in staining between MTST and thioflavine S was more pronounced in these cortical fields than in the hippocampus, especially for NFT (frontal cortex: about 82 %; temporal cortex: about 71 %; Table 1). The number of SP was also significantly higher with MTST than with Globus (29 %) (see Table 1). The Gallyas technique (Fig. 3A) appears to be slightly more sensitive than the Globus for NFT counting (28.6 ± 5.4 and 23.4 ± 4.6; $P < 0.025$) because the former technique stains mostly NFT and neuropil threads but does not label SP. We found that the staining of specific lesions with Campbell-Switzer-Martin is less homogeneous than the Gallyas method (Fig. 3C) and is not reliable for semi-automatic quantification analysis. In Fig. 1 (A and B), a non-significant difference in SP and NFT densities is observed between the two thioflavine S methods when counted using a reticule. However, the differentiation between stained structures and background is so greatly improved on MTST-stained sections that semi-automatic quantification by a computerized image analysis system is possible. Results obtained by semi-automatic analysis of MTST-treated materials were comparable to those obtained with the reticule and do not require a fastidious and time-consuming microscopic monitoring.

Fig. 1A–D. Comparison between classical thioflavine S method (**A**) and the modified thioflavine S technique (MTST) (**B**) and between Globus (**C**) and MTST (**D**). Note the optimal anatomical definition of senile plaques (SP) and neurofibrillary tangles (NFT) on MSTS-stained materials, and the increased signal-to-noise ratio of MTST as compared to classical thioflavine S. Subiculum (**A, B**) and CA3 (**C, D**). Scale bar (on C) = 100 μm

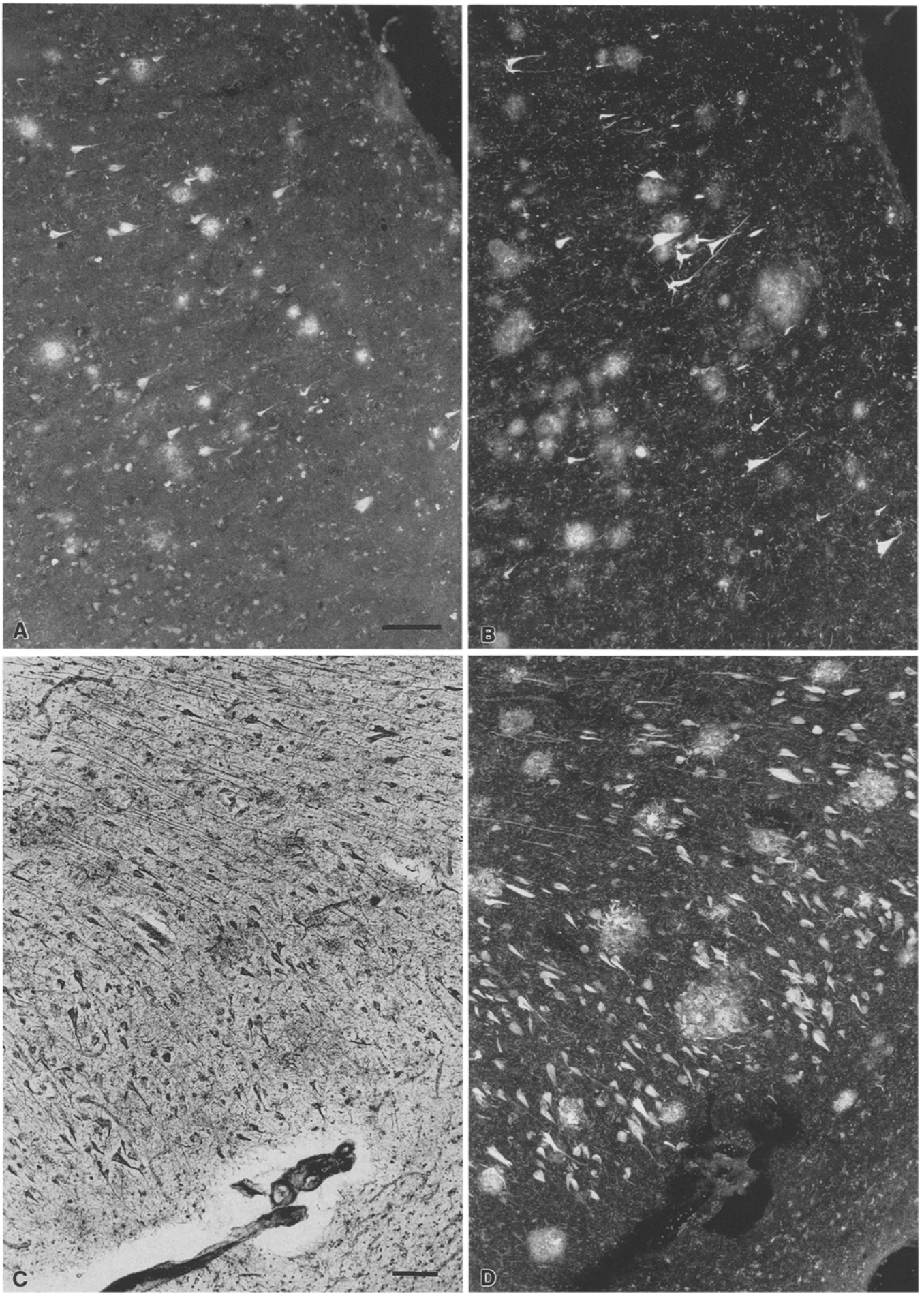


Fig. 1 A–D

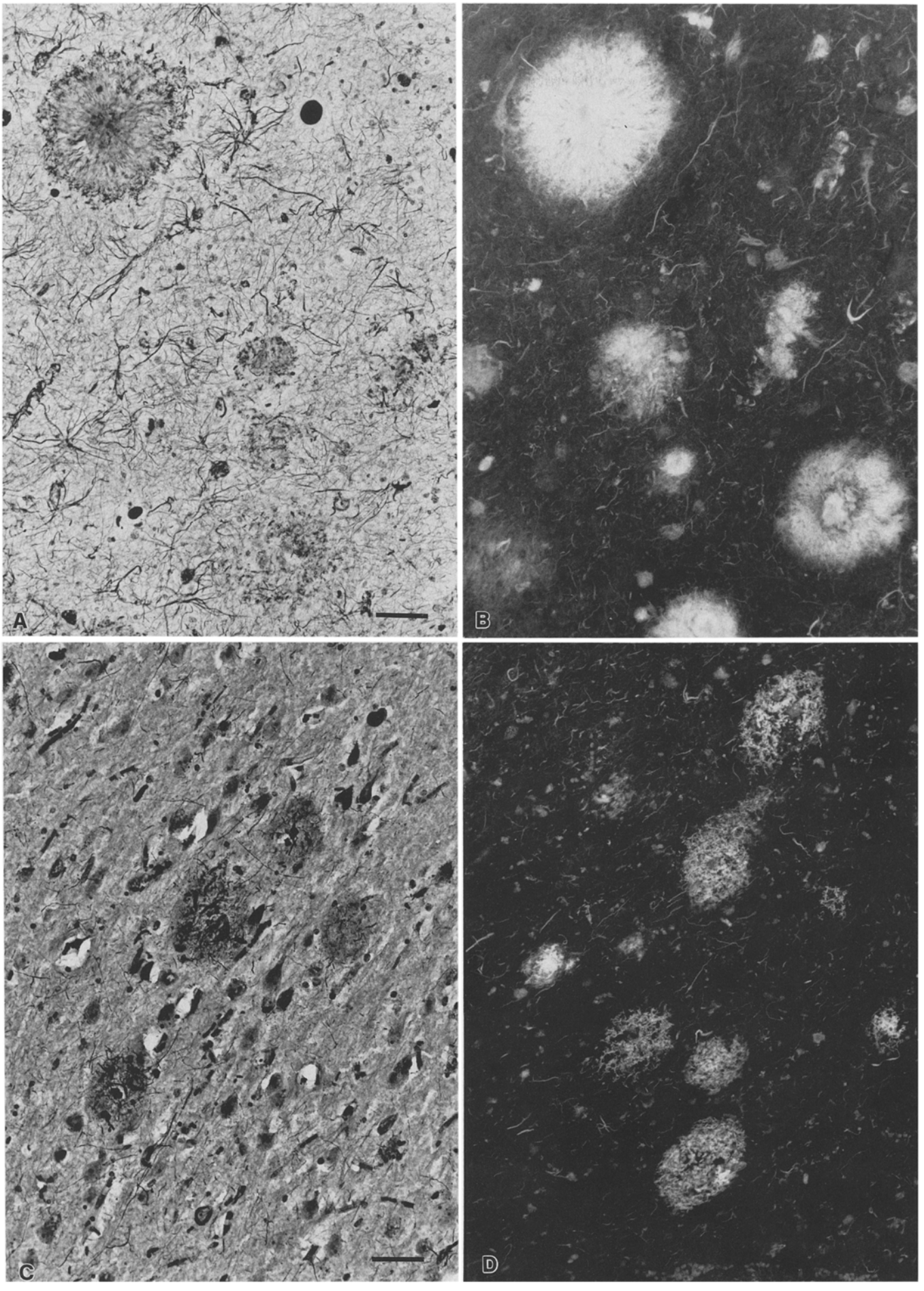


Fig. 2 A–D (for legend see next page)

Table 1. Comparison of number of neurofibrillary tangles (NFT) and senile plaques (SP) counted in adjacent fields from three different brain areas

Hippocampus (CA1, CA3 and Subiculum									
NFT					SP				
MTST	34.4 ± 14.2	vs	ThioS	34.8 ± 14.2	MTST	23.9 ± 3.4	vs	ThioS	21.3 ± 3.4
MTST	47.3 ± 8.6	vs	Glob.	24.9 ± 5.5*	MTST	24.6 ± 4.9	vs	Glob.	13.5 ± 3.4*
MTST	32.6 ± 3.4	vs	Gall.	28.9 ± 4.2	MTST	17.0 ± 2.0	vs	Biel.	7.0 ± 1.8*
MTST	69.0 ± 4.4	vs	CMS	39.2 ± 2.4*	MTST	19.2 ± 1.5	vs	CSM	14.2 ± 3.0*
Glob.	23.4 ± 4.6	vs	Gall.	28.6 ± 5.4	Glob.	9.4 ± 2.9	vs	Biel.	5.8 ± 1.3**
MTST	47.7 ± 4.5	vs	anti-tau	40.7 ± 3.9	MTST	26.1 ± 4.8	vs	Anti-A4	26.3 ± 4.4
Frontal Cortex (Area 10)									
MTST	12.8 ± 2.6	vs	ThioS	2.2 ± 0.6*	MTST	7.6 ± 0.8	vs	ThioS	3.2 ± 0.4*
MTST	22.3 ± 3.4	vs	Gall.	21.4 ± 3.5	MTST	14.0 ± 1.4	vs	Glob.	10.0 ± 1.2*
Temporal cortex (Areas 20, 21)									
MTST	18.8 ± 1.5	vs	ThioS	5.4 ± 1.1*	MTST	7.5 ± 1.2	vs	ThioS	6.5 ± 0.8
MTST	25.9 ± 1.7	vs	Gall.	23.7 ± 1.9	MTST	14.2 ± 1.9	vs	Glob.	10.9 ± 1.4*

Results represent means ± SEM from ten surveyed fields from a series of sections from six brains with Alzheimer's disease. Data from frontal and temporal cortices wee obtained in one brain. Comparable observations were made in other cases as well as in other frontal and temporal areas (see methods)
MTST: New modified thioflavine S technique; ThioS: classical thioflavine S method; Glob.: Globus; Biel.: Bielschowsky; Gall.: Gallyas; CSM: Campbell-Switzer-Martin
* Significantly different from MTST $P < 0.01$; ** Significantly different from Globus $P < 0.01$; paired two-sided Student's t-test

However, in addition to routine neuropathological case documentation, valuable quantitative data can be recorded at the same time.

Only minimal fading problems were encountered with the new method as compared to classical thioflavine S and the intensity of the specific fluorescence could be preserved for several months if sections are kept at 4 °C. The pretreatment with $KMnO_4$ and NaOH totally removes lipid autofluorescence resulting in an improved definition of the pathological lesions [16, 17]. Results were comparable for short or long fixation periods (for up 40 years).

In a few cases with severe gliosis, some reactive astrocytes were occasionally stained with MTST and were predominantly located in the superficial part of layer I and in the white matter. Staining of the glial element never influenced in quantification protocol. Diffuse amyloid deposits are also evidenced in hippocampal cortex as well as amyloid accumulation in the wall of small blood vessels [18]. The intense staining of neuropil threads (Fig. 2B) may be another advantage of this new method.

No statistical differences were obtained between the number of NFT and SP by using MTST and anti-tau or anti-β protein A4, respectively (Fig. 4A–C).

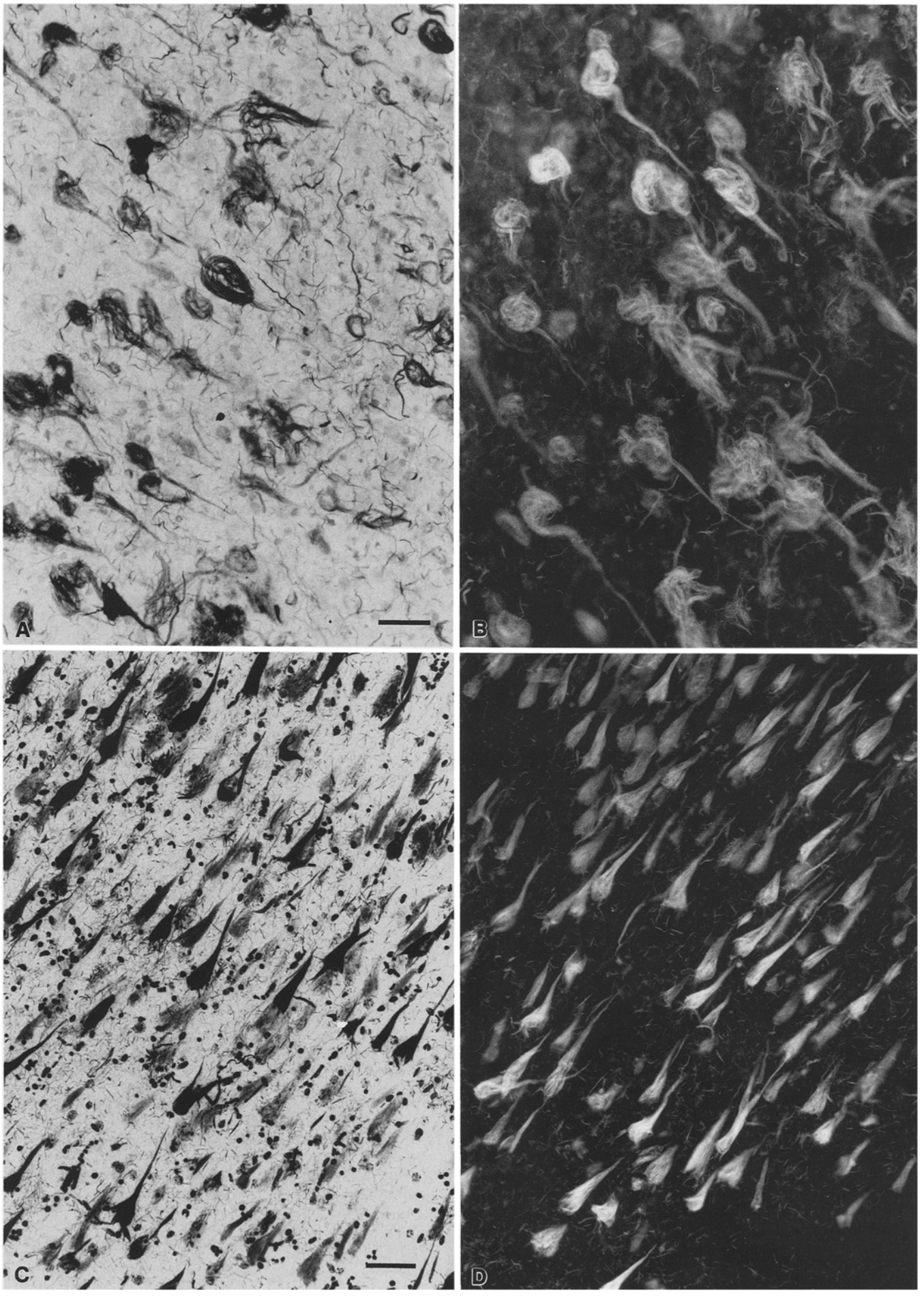
The time consumption of the MTST protocol is slightly increased (about 1 h) in comparison to classical thioflavine S, but is shorter than the immunostaining techniques and argentic impregnations which requires up to 2 days. However, discrimination of specific lesions are so highly improved that time consumption for careful microscopic observation is considerably reduced and semi-automatic analysis is easy to perform. Moreover, the MTST is 5 to 100 times less expensive than any of the other methods.

Discussion

The present study reveals the discrepancy between staining patterns obtained using the following methods: Bielschowsky, Campbell-Switzer-Martin, Globus, Gallyas, both thioflavine S methods and two immunostaining techniques. The MTST appears to give the best results for the demonstration of SP and NFT in AD. In hippocampus, this method permits the visualization of as much as 60 % more SP than the classical Bielschowsky argentic impregnation, and 47 % more NFT than the Globus technique. Immunohistochemical methods gave results comparable to MTST but are more expensive and

Fig. 2A–D. Comparison of SP staining between argentic impregnations [Globus (**A**) and Bielschowsky (**C**)] and MTST (**B, D**) on adjacent sections. There are higher SP densities on the MTST-stained sections. Both pairs of microphotographs are from the CA3 field of the hippocampus. Scale bars (**A** and **C**) = 50 μm

Fig. 3A–D. Comparison of NFT staining between argentic impregnations [Gallyas (**A**) and Campbell-Switzer-Martin (**C**)] and MTST (**B, D**) on adjacent sections. There are quite similar NFT densities on the MTST-stained sections; however, the definition of NFT is enhanced on MTST-stained sections. Both pairs of microphotographs are from the subiculum of the hippocampus. Scale bars (**A** and **C**) = 20 μm


A
B
C
D

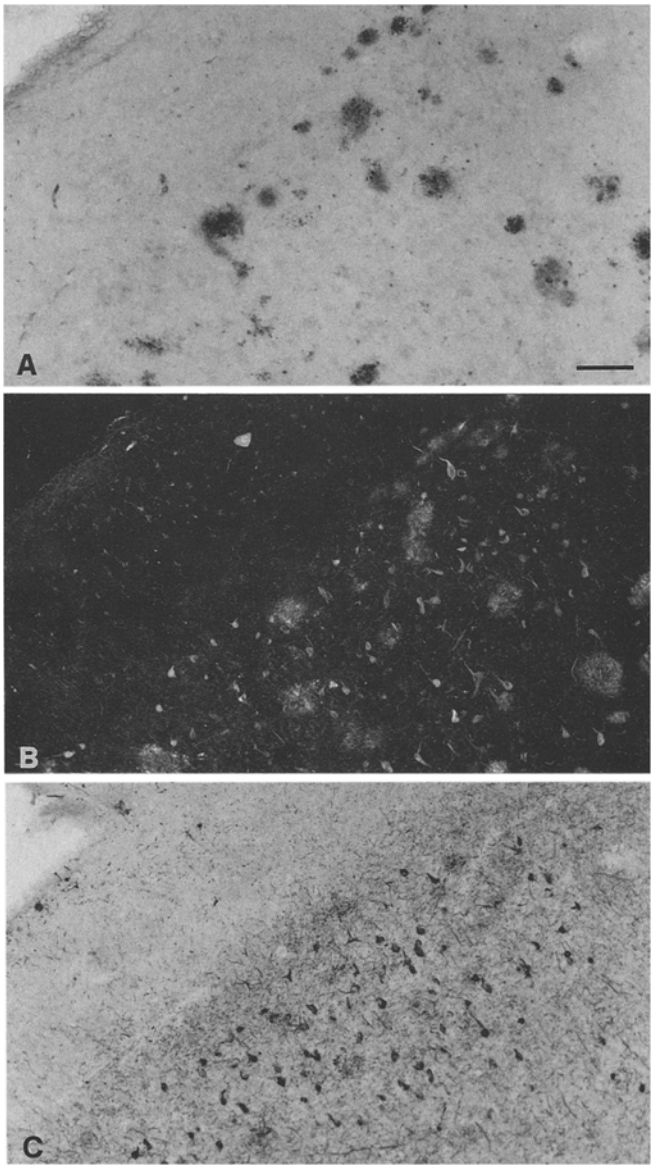


Fig. 4A–C. Comparison of immunostaining for amyloid β protein A4 (**A**) and tau protein (**C**) with MTST (**B**). There is a comparable number of SP and NFT. Note that the anti-tau antibody lightly stains the SP as well as neuropil threads. Subiculum. Scale bar (**A**) = 50 μm

time consuming, although this approach is now currently used to demonstrate SP and NFT [2, 9, 13, 28, 44, 45]. According to Wisniewski et al. [44] the Bielschowsky and immunostaining methods revealed similar number of SP.

A recent survey addressed to 261 neuropathologists [43] about the histological diagnosis of AD showed that the main discrepancy between data on the density of NFT and SP arises from the type and quality of staining techniques. In addition the quantification of the AD lesions were not performed with a standardized protocol. Only 21% of respondents were following the specific quantitative criteria proposed by Khachaturian [31]. Our present data as well as observations by other investigators [9, 13, 28, 33] confirm that significant differences can be observed depending on the staining method. Differences in the pathological diagnosis of AD were also evident in a recent overview by Duyckaerts et al. [10]. It is worth noting that the classical thioflavine S method may be more sensitive to tissue preservation than MTST, since in neocortical areas of some cases we observed higher NFT densities with MTST (see Table 1). This suggest that the pretreatment may be useful to reveal the full NFT staining pattern. Thus, MTST [16, 18] offers a convenient protocol for routine neuropathological diagnosis as reliable as immunohistochemical methods. Also, MTST could be used as a rapid protocol on floating deparaffinized or frozen sections and may be adequate to develop standard criteria for AD diagnosis.

The differences in staining of SP and NFT observed between different methods indicate that a more accurate definition of the existing criteria for AD diagnosis should be proposed. Moreover, the fact that this improved thioflavine S technique can be used to visualize neuropil threads [5], amyloid deposits, vascular lesions, indicates that new criteria may be added to those currently used for AD diagnosis. In view of the differences in NFT and SP distribution between cytoarchitectonic areas [1, 15, 21, 22, 24–27, 34, 37, 39, 40] criteria should also rely on a precise laminar and regional quantitative analysis. Such an analysis is easy and rapid to perform using the MTST method [21, 26].

In conclusion, the use of our modification of the thioflavine S method permits rapid and reliable quantitative assessments of histopathological diagnosis of AD and, therefore, is useful for further detailed neuropathological investigations.

Acknowledgements. The authors thank M. Surini, I. Mikolajewski, B. Greggio, A. J. Schoeb and P. Y. Vallon for expert technical assistance.

References

1. Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW (1991) The topographical and neuroanatomical distribution of neurofibrillary tangles and neurite plaques in the cerebral cortex of patients with Alzheimer's disease. Cerebral Cortex 1:103–116
2. Behrouz N, Défossez A, Delacourte A, Hublau P, Mazzuca M (1989) Alzheimer's disease: glycolytic pretreatment dramatically enhances immunolabeling of senile plaques and cerebrovascular amyloid substance. Lab Invest 61:576–580
3. Benson DF, Davis RJ, Snyder BD (1988) Posterior cortical atrophy. Arch Neurol 45:789–793
4. Bielschowsky M (1904) Die Silberimprägnation der Neurofibrillen. J Psychol Neurol 3:169–188
5. Braak H, Braak E, Grundke-Iqbal I, Iqbal K (1986) Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: a third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. Neurosci Lett 65:351–355
6. Campbell SK, Switzer RC, Martin TL (1987) Alzheimer's plaques and tangles: a controlled and enhanced method. Soc Neurosci Abstr 13:678

7. Défossez A, Beauvillain JC, Delacourte A, Mazzuca M (1990) Alzheimer's disease: a new evidence for common epitopes between monotubule associated protein tau and paired helical filaments (PHF): demonstration at the electron microscope by a double immunogold labelling. Virchows Arch [A] 413:141–145
8. Delacourte A, Flament S, Dibe EM, Hublau P, Sablonnière B, Hémon B, Scherrer V, Défossez A (1990) Pathological proteins Tau 64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer's disease. Demonstration with a panel of antibodies against Tau proteins. Acta Neuropathol 80:111–117
9. Duyckaerts C, Brion JP, Hauw JJ, Flament-Durand J (1987) Quantitative assessment of the density of neurofibrillary tangles and senile plaques in senile dementia of the Alzheimer type. Comparison of immunocytochemistry with a specific antibody and Bodian's protargol method. Acta Neuropathol (Berl) 73:167–170
10. Duyckaerts C, Delaère P, Hauw JJ, Abbamondi-Pinto AL, Sorbi S, Allen I, Brion JP, Flament-Durand J, Duchen L, Kauss J, Schlote W, Lowe J, Probst A, Ravid R, Swaab DF, Renkawek K, Tomlinson B (1990) Rating of the lesions in senile dementia of the Alzheimer type: concordance between laboratories. A European multicenter study under the auspices of EURAGE. J Neurol Sci 97:295–323
11. Flament S, Delacourte A, Delaère P, Duyckaerts C, Hauw JJ (1990) Correlation between microscopical changes and tau 64 and 69 biochemical detection in senile dementia of the Alzheimer type. Tau 64 and 69 are reliable markers of the neurofibrillary degeneration. Acta Neuropathol 80:212–215
12. Gallyas F (1971) Siver staining of Alzheimer's neurofibrillary changes by means of physical development. Acta Morphol Acad Sci Hung 19:1–8
13. Gentleman SM, Bruton C, Allsop D, Lewis SJ, Polak JM, Roberts GW (1989) A demonstration of the advantages of immunostaining in the quantification of amyloid plaque deposits. Histochemistry 92:355–358
14. Globus JH (1927) The Cajal and Hortega glia staining methods. Arch Neurol Psychiatry 8:263–271
15. Golaz J, Bouras C, Hof PR (1991) Motor cortex involvement in presenile dementia: report of a case. J Geriatr Psychiatry Neurol (in press)
16. Guntern R, Bouras C, Vallet PG, Hof PR (1989) An improved staining method for senile plaques and neurofibrillary tangles in Alzheimer's disease: quantitative comparison with other techniques. Soc Neurosci Abstr 15:1038
17. Guntern R, Vallet PG, Bouras C, Constantinidis J (1989) An improved immunohistostaining procedure for peptides in human brain. Experientia 45:159–161
18. Guntern R, Bouras C, Hof PR, Vallet PG (1991) An improved staining method for senile plaques and neurofibrillary tangles in Alzheimer's disease. Experientia (in press)
19. Hauw JJ, Duyckaerts C, Delaère P (1988) Neuropathology of aging and DAT: how can age-related changes be distinguished from those due to disease processes. In: Henderson AS, Henderson JH (eds) Etiology of dementia of Alzheimer's type. John Wiley & Sons, Chichester, pp 195–211
20. Henderson VW, Finch CE (1989) The neurobiology of Alzheimer's disease. J Neurosurg 70:335–353
21. Hof PR, Bouras C (1991) Object recognition deficit in Alzheimer's disease: possible disconnection of the occipito-temporal component of the visual system. Neurosci Lett 122:53–56
22. Hof PR, Morrison JH (1990) Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease. II. Primary and secondary visual cortex. J Comp Neurol 301:55–64
23. Hof PR, Bouras C, Constantinidis J, Morrison JH (1989) Balint's syndrome in Alzheimer's disease: specific disruption of the occipito-parietal visual pathway. Brain Res 493:368–375
24. Hof PR, Bouras C, Constantinidis J, Morrison JH (1990) Selective disconnection of specific visual association pathways in cases of Alzheimer's disease presenting with Balint's syndrome. J Neuropathol Exp Neurol 49:168–184
25. Hof PR, Cox K, Morrison JH (1990) Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease. I. Superior frontal and inferior temporal cortex. J comp Neurol 301:44–54
26. Hof PR, Knabe R, Bovier P, Bouras C (1991) Neuropathological observations in a case of autism presenting with self-injury behavior. Acta Neuropathol (in press)
27. Hof PR, Perl DP, Loerzel AJ, Morrison JH (1991) Neurofibrillary tangles distribution in the cerebral cortex of Parkinson dementia cases from Guam: differences with Alheimer's disease. Brain Res (in press)
28. Ikeda SI, Allsop D, Glenner GG (1989) Morphology and distribution of plaque and related deposits in the brains of Alzheimer's disease and control cases. Lab Invest 60:113–122
29. Jagust WJ, Davies P, Tillerborcich JK (1990) Focal Alzheimer's disease. Neurology 40:14–19
30. Katzman R, Saitoh T (1991) Advances in Alzheimer's disease. FASEB J 5:278–286
31. Khachaturian ZS (1985) Diagnosis of Alzheimer's disease. Arch Neurol 42:1097–1104
32. Kim KS, Miller DL, Sapienza VG, Chen CJ, Vai C, Grundke-Iqbal I, Curry JR, Wisniewski HM (1988) Production and characterization of monoclonal antibodies reactive to synthetic cerebrovascular amyloid peptide. Neurosci Res Commum 2:121–130
33. Lamy C, Duyckaerts P, Delaère P, Payan CH, Fermanian J, Poulain V, Hauw JJ (1989) Comparison of seven staining methods for senile plaques and neurofibrillary tangles in a prospective series of 15 elderly patients. Neuropathol Appl Neurobiol 15:563–578
34. Lewis DA, Campbell MJ, Terry RD, Morrison JH (1987) Laminar and regional distribution of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: a quantitative study of visual cortex and auditory cortices. J Neurosci 7:1799–1808
35. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of the Department of health and Human services task Force on Alzheimer's disease. Neurology 34:939–944
36. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L and CERAD neuropathologists (1991) The Consortium to establish a registry for Alzheimer's disease (CERAD). II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41:479–486
37. Mutrux S (1947) Diagnostic différentiel histologique de la maladie d'Alzheimer et de la démence sénile – Pathophobie de la zone de projection corticale. Monatsschr Psychiatr Neurol 113:100–117
38. Pappola MA, Omar RA, Robakis NK (1991) Immunohistochemical demonstration of antioxidant enzymes in Alzheimer's disease. Am J Pathol (in press)
39. Pearson RCA, Esiri MM, Hiorns RW, Wilcock GK, Powell TPS (1985) Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer's disease. Proc Natl Acad Sci USA 82:4531–4534
40. Rogers J, Morrison JH (1985) Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease. J Neurosci 5:2801–2808
41. Rudelli RD, Ambler MV, Wisniewski HM (1984) Morphology and distribution of Alzheimer neuritic (senile) and amyloid plaques in striatum and diencephalon. Acta Neuropathol (Berl) 64:273–281
42. Wilcock GK, Esiri MM (1982) Plaques, tangles and dementia. A quantitative study. J Neurol Sci 56:343–356

43. Wisniewski HM, Rabe A, Zigman W, Silverman W (1989) Neuropathological diagnosis of Alzheimer disease. J Neuropathol Exp Neurol 48:606–609
44. Wisniewski HM, Wen GY, Kim KS (1989) Comparison of four staining methods on the detection of neuritic plaques. Acta Neuropathol 78:22–27
45. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Harigaya Y (1988) Diffuse type of senile plaques in the brains of Alzheimer-type dementia. Acta Neuropathol 77:113–119
46. Yamamoto T, Hirano A (1986) A comparative study of modified Bielschowsky, Bodian and thioflavine S stains on Alzheimer's neurofibrillary tangles. Neuropathol Appl Neurobiol 12:3–9