An improved thioflavine S method for staining neurofibrillary tangles and senile plaques in Alzheimer's disease

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Abstract. Large differences are usually observed when standard staining methods for a number of pathological lesions in neurodegenerative disorders are compared. With the modified thioflavine S method presented here (easy and cheap to perform), the morphological appearance of the stained neurofibrillary tangles (NFT) and senile plaques (SP) is greatly improved. Furthermore, the intense contrast between stained lesions and background obtained with this technique permits an accurate automatic quantification of NFT and SP using a computer-assisted image analysis system.
Key words. Senile plaques; neurofibrillary tangles; Alzheimer's disease; thioflavine S.

Alzheimer's disease (AD), the major cause of dementia in adults, is characterized clinically by impairments in memory, language and visuospatial skills, and modifications of behavior. Although clinical criteria can be used to make a diagnosis of probable AD[1], the histological demonstration of senile plaques (SP) and neurofibrillary tangles (NFT) is still necessary to confirm the diagnosis[2]. The sensitivity of several staining methods (Bielschowsky, thioflavine S, Congo red, Globus and Bodian, and the immunostaining of β-amyloid) has previously been evaluated[3–9]. The modified thioflavine S technique (MTST) described here allows for the visualization of as many as 60% more SP than the classical thioflavine S method[18], and can be used routinely to confirm a clinical diagnosis of AD as well as for detailed quantitative neuropathological investigations.

Material and methods

Six brains from patients with clinically diagnosed and neuropathologically confirmed Alzheimer's disease (79 ± 7.7 years old) were used in the present study. Tissues were obtained at autopsy (post mortem delay up to 36 h), fixed by immersion in 15 vol.% formalin for 30 days and then stored in 5 vol.% formalin for up to 40 years (range 2 to 40). The appropriate hippocampal regions were selected 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 histochemical procedure for the MTST was as follows. 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 all steps, 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 mounted under a coverslip with glycerin-H_2O (3:1). 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 in glycerin-H_2O (3:1)[10].

Deparaffinized floating sections (30 µm) can also be stained with MTST using the same protocol.

For the semi-automatic quantification of the lesions, 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) was used. For the automatic counting of 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. Although the time consumption for the technician is increased (about 1 h), discrimination of specific lesions is so highly improved that the time consumption for careful microscopic observation is considerably reduced and automatic analysis is easy to perform.

Results and discussion

The modified thioflavine S technique (MTST) gives excellent results for the demonstration of both SP and NFT (figs 1–4). The difference between MTST and thioflavine S was more pronounced in the cortical fields as compared to the hippocampus, especially for NFT. In figures 1 and 2 (hippocampal area), a small but non-significant difference in SP and NFT densities is observed

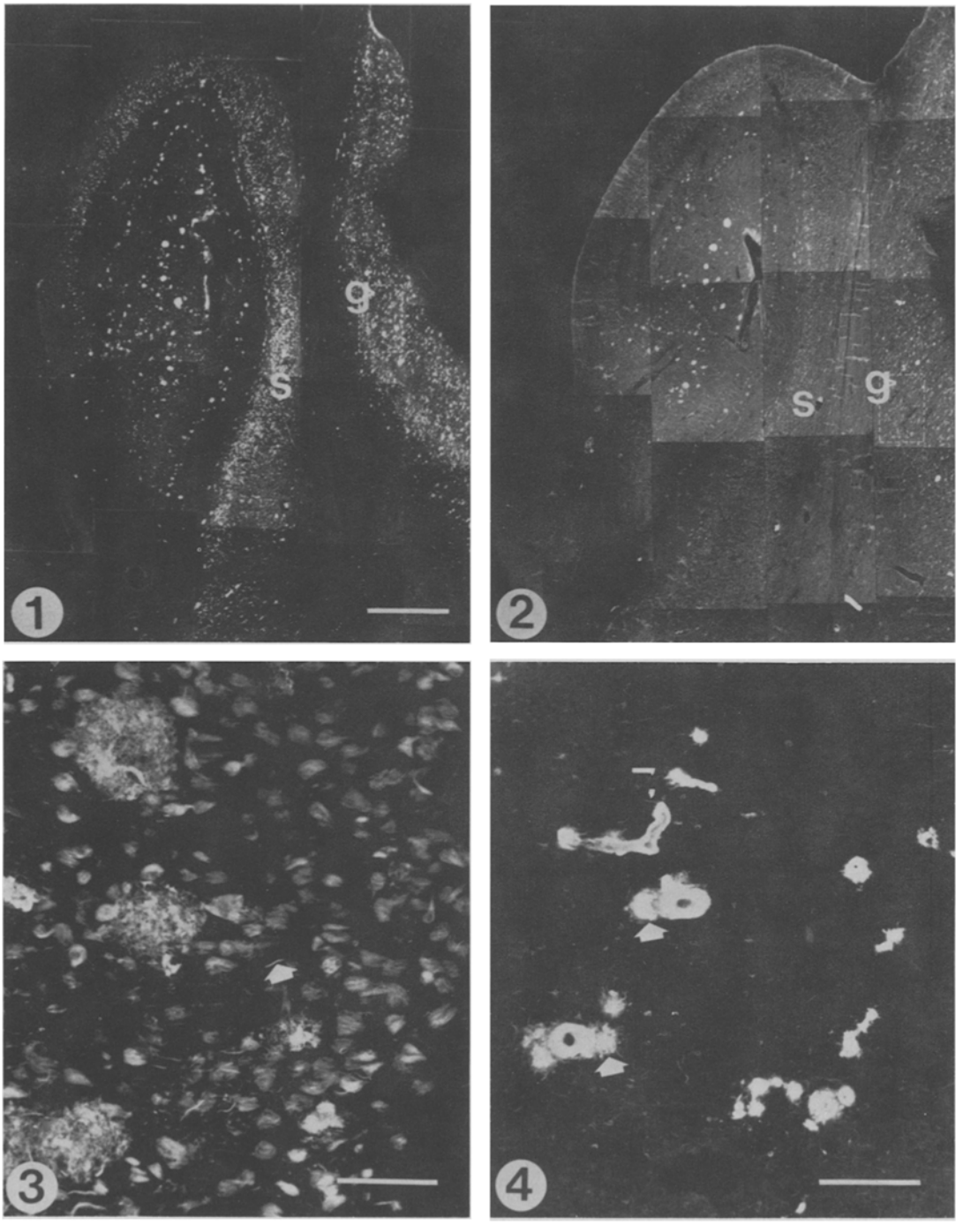

Figure 1. Photomontage of the hippocampus stained with MTST in an Alzheimer's disease case. Note the very high density of NFT and SP in subiculum (s) and gyrus parahippocampalis (g). The high contrast between lesions and background is evidenced in comparison with the adjacent section shown in fig. 2. (bar: 1000 µm)

Figure 2. Adjacent photomontage of the hippocampus shown in fig. 1 stained with the classical thioflavine S method. Only a few SP and NFT are visualized, owing essentially to the high background staining and the low morphological definition of the lesions.

Figure 3. SP and NFT stained with MTST in hippocampal gyrus. Arrow indicates a neuropil thread. (bar: 50 µm)

Figure 4. Amyloid deposition in the wall of the small blood vessels in the neocortex and around vessels (arrows). (bar: 100 µm)

between the two thioflavine methods when counted by the unaided eye. However, the contrast between stained structures and background is so greatly enhanced in MTST stained sections that semi-automatic quantification by a computerized image analysis system is possible and does not require fastidious and time-consuming microscopic monitoring. Only minimal fading problems were encountered with the new method as compared to classi-

cal thioflavine S, and the intensity of the specific fluorescence could be preserved for several months if sections were kept at 4 °C. The pretreatment with $KMnO_4$ and NaOH totally removes lipid autofluorescence, resulting in an improved definition of the pathological lesions[11,12]. Results were comparable for short or long fixation periods.

Reactive astrocytes are occasionally stained with MTST and are predominantly located in the superficial part of layer I and in the white matter. Diffuse amyloid deposits are also evidenced in the hippocampal cortex, as well as amyloid accumulation in the walls of several small blood vessels (fig. 4). The intense staining of neuropil threads (fig. 3) may be another advantage of this new method. The modified thioflavine S technique (MTST) presented here may be very convenient for routine neuropathological diagnoses and, on floating deparaffinized or frozen sections, could be used as a rapid standard protocol for developing reliable criteria for AD diagnosis.

Moreover, the fact that this improved thioflavine S technique can be used to visualize neuropil threads[13], amyloid deposits, and vascular lesions, indicates that new criteria may be added to those currently used for AD diagnosis[14–17].

In conclusion, the use of our modification of the thioflavine S method permits rapid and reliable quantitative assessments of histopathological changes resulting from AD and could therefore be useful for further detailed neuropathological investigations.

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Photon emission from normal and tumor human tissues

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Abstract. Photon emission in the visible and near ultraviolet range by samples of human tissue removed during surgery has been measured by means of a low noise photomultiplier coupled to a data acquisition system. The results show that among the 25 analyzed samples the 9 from normal tissues had an emission rate of the order of some tens of photons/cm^2 min, while most of the 16 tumor tissue samples had a very much higher rate.
Key words. Ultraweak radiation; cancer; low level luminescence; biophoton.

The emission of radiation from living systems was suggested at the beginning of this century by G. Gurwitsch[1], to explain some experimental observations on duplicating cells. The presence of this radiation was revealed in Italy forty years ago by Facchini et al.[2,3], who discovered that living systems emit light at a very low level. Although a number of scientists in the Soviet Union[4] and in Eastern countries[5,6] continued to work on photon emission from biological systems, it is only in the last few years that some interest in the problem has been growing in Western countries[7–10].

The original Gurwitsch hypotheses suggested that living systems transmit and receive information by means of electromagnetic waves. This statement is at present controversial and neither the experiments nor their interpretation are conclusive[11]. It is widely accepted, however, that the intensity of the emitted radiation is closely connected with the biochemical processes occurring in a sys-