*Neuroscience Letters*, 82 (1987) 246–252
Elsevier Scientific Publishers Ireland Ltd.

NSL 04912

# Quantitative autoradiography of hippocampal $GABA_B$ and $GABA_A$ receptor changes in Alzheimer's disease

Dorothy C.M. Chu, John B. Penney Jr. and Anne B. Young

*Department of Neurology and the Neuroscience Program, University of Michigan, Ann Arbor, MI 48109 (U.S.A.)*

(Received 17 February 1987; Revised version received 25 June 1987; Accepted 8 July 1987)

*Key words:* Hippocampus; Dementia; Human; γ-Aminobutyric acid (GABA) receptor; Baclofen

$GABA_B$ and $GABA_A$ receptors were examined by quantitative [$^3$H]GABA autoradiography in postmortem human hippocampus from 6 histopathologically verified cases of dementia of the Alzheimer type (DAT) and 6 normal controls. Significant decrements in the $B_{max}$ for both types of GABA receptors were observed in DAT hippocampus as compared to normal controls. No significant differences in $K_d$ values were revealed. As compared to controls, DAT hippocampus exhibited fewer $GABA_B$ receptors in stratum moleculare of the dentate gyrus, stratum lacunosum-moleculare and stratum pyramidale of $CA_1$. Significant loss of $GABA_A$ receptors in DAT hippocampus was also observed in the $CA_1$ pyramidal cell region. These changes could not be correlated with differences in age nor in postmortem delay between the two groups. These findings may reflect the neuronal pathologies in $CA_1$ region, in dentate gyrus, and in projections from the entorhinal cortex which are associated with the memory impairment in DAT.

The pathologic hallmarks of dementia of the Alzheimer type (DAT) are most strikingly evident in the hippocampus. Abundant neurofibrillary tangles, neuritic plaque formation [7] and widespread granulovacuolar degeneration are localized in specific cell layers of the hippocampus [1]: the $CA_1$ pyramidal fields, entorhinal cortical laminae I and IV, and the subiculum. Degeneration of these neuronal elements disrupts the multisynaptic circuitry through the hippocampus and serves to isolate the hippocampus from its association and limbic connections [9]. This functional dissociation of the hippocampus may contribute to the impaired memory formation and retrieval seen in dementia of the Alzheimer type (DAT) [20].

γ-Aminobutyric acid (GABA) is an ubiquitous inhibitory neurotransmitter of intrinsic hippocampal non-pyramidal neurons [21]. Several findings implicate the participation of GABAergic systems in DAT pathology. The presence of GAD-positive immunoreactive terminals in senile plaques suggests that these may represent remnants of degenerating GABAergic processes [22]. The findings of reduced synap-

*Correspondence:* A.B. Young, 1103 E. Huron Street, Neuroscience Laboratory Building, Ann Arbor, MI 48104, U.S.A.

tosomal GABA uptake in hippocampus and cortex of Alzheimer brains are indicative of loss of GABAergic terminals [8]. GABA is also widely co-localized with somatostatin [19], the markers for which are consistently reduced in DAT [18]. Cerebrospinal fluid (CSF) concentrations of GABA are notably lower in DAT patients as compared to controls [23].

GABA exerts synaptic effects via two types of receptors [3]. In hippocampal granule or pyramidal cell somata, GABA elicits fast inhibitory postsynaptic potentials (IPSPs) by increasing chloride influx [2]. These somatic GABAergic responses are mediated by bicuculline-sensitive $GABA_A$ receptors. Within the dendritic and somatic layers of granule and pyramidal cells, GABA can also cause a bicuculline-insensitive later- onset IPSP by opening $K^+$ channels [5]. This effect of GABA can be mimicked by baclofen and involves activation of a distinct $GABA_B$ receptor.

Previously we have described losses of baclofen-sensitive $GABA_B$ receptors in the frontal cortex of persons who died with Alzheimer's disease [4]. In the present study, using quantitative autoradiography, we have compared the distribution and numbers of $GABA_A$ and $GABA_B$ receptors in the hippocampus of individuals who died with DAT and of normal controls who died without neurologic disease. The DAT patients died with respiratory complications following 6 or more years of progressive intellectual decline and illness. The control cases suffered sudden cardiac arrest or trauma. Previous drug history indicated that one DAT patient was taking haloperidol (6 mg/day), another was taking benzodiazepines, and one of the control subjects was taking a calcium channel blocker. At autopsy, the brain was removed and halved in the sagittal plane. One half was fixed in formalin for histopathologic identification and the other half was retained at $-70°C$ for receptor binding assays. The diagnosis of Alzheimer's disease was based on previously defined neuropathologic criteria [11]. Blocks of hippocampus from 6 normal control and 6 DAT brains were obtained, coded and stored at $-70°C$ until use. Mean age and postmortem delay (PMD) were not significantly different between the two groups (mean control age $= 55 \pm 18$ years, mean DAT age $= 71 \pm 7$ years, mean control PMD $= 15.5 \pm 7.3$ h, mean DAT PMD $= 18.6 \pm 9.8$ h, both non-significant by independent Student's $t$-test). The DAT brains were atrophic (weighing less than 1300 g). Silver-stained sections of DAT hippocampus exhibited more than 10 tangles and/or plaques per high-power field.

Tissue blocks were equilibrated at $-20°C$ one day before the assay was performed. Forty-four 20-$\mu$m-thick sections were cut at $-20°C$ from each block. Sections were thaw-mounted onto gelatin/chrom–alum-coated slides and allowed to air-dry. Slides were given a 15-min prewash in 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$ (pH 7.40 at 4°C) to remove endogenous GABA, and dried under a stream of cool air. Each slide was then placed in a vial containing [$^3$H]GABA (Amersham, 50 Ci/mmol, Batch 35) with the above buffer and various drug displacers. For $GABA_B$ binding, 10 $\mu$M isoguvacine (Cambridge Research Biochemicals, Cambridgeshire, U.K.) was present to block binding to $GABA_A$-sensitive sites. This concentration of isoguvacine was determined to be optimal for blocking $GABA_A$ binding sites without affecting $GABA_B$ receptor binding. For $GABA_A$ binding, 100 $\mu$m ($\pm$)-baclofen (kindly donated by CIBA-GEIGY Corporation, Summit, NJ) was present to block

binding to $GABA_B$-sensitive sites. Non-specific [$^3$H]GABA binding was assessed in the presence of both 100 $\mu$M ($\pm$)-baclofen and 100 $\mu$M isoguvacine, to ensure that both $GABA_B$ and $GABA_A$ binding sites were adequately blocked.

Studies of saturation binding to $GABA_B$ and $GABA_A$ receptors were conducted using the method of isotopic dilution of [$^3$H]GABA by non-radioactive GABA to achieve a range of free GABA concentrations ranging from 6 nM to 1 $\mu$M. $GABA_B$ and $GABA_A$ receptor assays each employed 22 sections from each brain examined. This permitted 11-point saturation curves with blanks at each concentration point. The binding assay was conducted at 4°C for 45 min. After this period slides were removed individually and subjected to two quick rinses with cold buffer, followed by one rinse with 1.25% glutaraldehyde in acetone, and immediately dried. Slides were mounted in an X-ray cassette with the appropriate radioactive standards and apposed to LKB tritium-sensitive $^3$H-Ultrofilm for 3 weeks at 4°C. The standards employed are $^{14}$C-plastic standards that were previously calibrated against brain paste standards containing known amounts of tritium and protein [15]. Films were developed in Kodak D19, fixed and dried. Optical film densities in various regions of interest were quantified by spot densitometry relative to the known standards. The regions of interest included stratum moleculare of dentate gyrus, stratum lacunosum-moleculare of $CA_1$, stratum pyramidale of $CA_1$ and subiculum. The autoradiographic image was compared to a Cresyl violet stain of the underlying tissue section to determine the anatomic regions of interest. Bound values at each concentration of GABA in these various regions were quantified in pmol/mg protein. For both types of GABA receptors, Scatchard plots were constructed to generate $B_{max}$ and $K_d$ values using computer-assisted linear regression. The code was broken and differences in $B_{max}$ and $K_d$ values between control and DAT groups were analyzed for significance by independent, two-tailed Student's $t$-test and by Mann-Whitney $U$-test.

In the presence of 2.5 mM $CaCl_2$, [$^3$H]GABA bound to human hippocampus in a distinct laminar pattern to two populations of receptors – isoguvacine-sensitive $GABA_A$ and baclofen-sensitive $GABA_B$ receptors. When both isoguvacine and baclofen were present, no specific [$^3$H]GABA binding remained on the section. The distributions of $GABA_B$ and $GABA_A$ receptor binding to human hippocampus were most prominent in the stratum moleculare of the dentate gyrus, stratum lacunosum-moleculare and stratum pyramidale of $CA_1$. Intermediate density of [$^3$H]GABA binding to the subiculum and dentate hilus, and negligible binding to white matter were observed (Fig. 1). There were roughly 3 times more $GABA_A$ than $GABA_B$ receptors in both control and DAT hippocampus.

Specific binding of [$^3$H]GABA to both $GABA_B$ and $GABA_A$ receptor sites was saturable after 45 min at 4°C. Analysis of the Scatchard plots did not disclose consistent differences in the $K_d$ values between controls and DATs, but did reveal striking changes between the two groups in the $B_{max}$ of hippocampal [$^3$H]GABA binding. Notably, $GABA_B$ receptors in the DAT group were reduced by 50–70% relative to controls in stratum moleculare of dentate gyrus, stratum lacunosum-moleculare and stratum pyramidale of $CA_1$. Significant differences in $B_{max}$ values of $GABA_A$ binding between DAT and controls were found only in stratum pyramidale of $CA_1$. No sig-

nificant difference in $B_{max}$ values of $GABA_B$ nor $GABA_A$ binding was seen in the subiculum (Fig. 2).

The overlapping distribution in $GABA_B$ and $GABA_A$ receptors in human hippocampus may indicate that the targets of $GABA_B$ and $GABA_A$ innervation are similar.

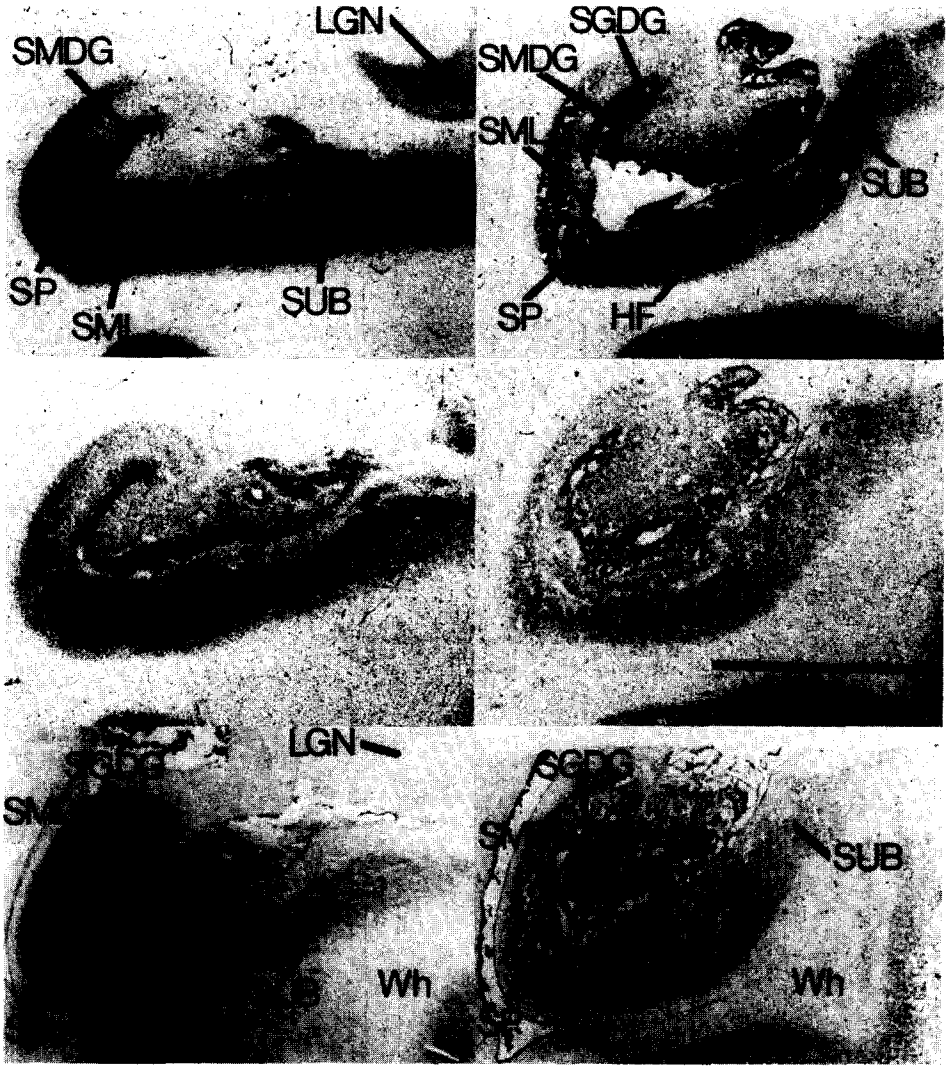


Fig. 1. Representative autoradiograms of [$^3$H]GABA (20 nM) binding to human hippocampal sections from a control (left) and a DAT (right) brain. $GABA_A$ binding (top) was conducted in the presence of 100 $\mu$M ($\pm$)-baclofen to block binding of [$^3$H]GABA to $GABA_B$ sites. Conversely, $GABA_B$ binding (middle) was conducted in the presence of 10 $\mu$M isoguvacine to block binding to $GABA_A$ sites. The corresponding Nissl-stained sections are shown on the bottom row. SMDG, stratum moleculare of dentate gyrus; SGDG, stratum granulosum of dentate gyrus; SML, stratum lacunosum-moleculare of $CA_1$; SP, stratum pyramidale of $CA_1$; SUB, subiculum; HF, hippocampal fissure; Wh, white matter; LGN, lateral geniculate nucleus. Bar = 0.5 cm.

The same local circuit GABAergic interneuron may elict postsynaptic membrane hyperpolarization via both $GABA_A$ and $GABA_B$ receptors. That there appear to be relatively more $GABA_A$ than $GABA_B$ receptors in hippocampus is also consistent with the electrophysiologic observation that $GABA_A$-mediated mechanisms predominate in the hippocampus [5, 13]. Interestingly, the distribution of $GABA_A$ receptors in human hippocampus is in excellent agreement with that of benzodiazepine receptors [12]. Both show highest density of binding in dentate molecular layer and $CA_1$ pyramidal layer. The distribution of GABA receptors in hippocampus is also in accordance with both the topography of GAD-positive [14] and GABA-immunoreactive terminals [6], and that of somatostatin receptors [17].

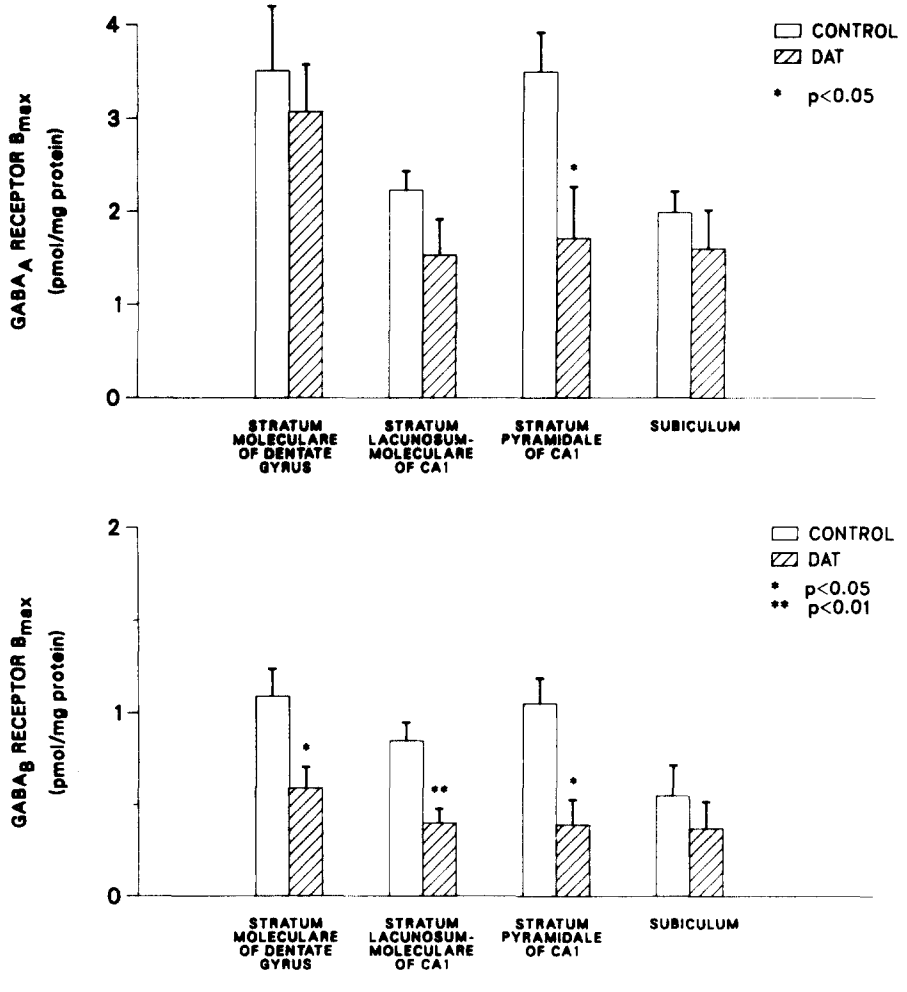


Fig. 2. Histogram of $B_{max}$ values of $GABA_A$ (top) and $GABA_B$ (bottom) binding to control (open bars) and DAT (hatched bars) hippocampus. The mean $B_{max}$ values and S.E.M. values in pmol/mg protein represent the average of 6 control and 6 DAT subjects. Significant differences were determined by independent, two-tailed Student's $t$-statistic and by Mann-Whitney $U$-test.

The decreased density of $GABA_B$ and $GABA_A$ receptors in stratum lacunosum-moleculare and stratum pyramidale of $CA_1$ region in DAT hippocampal formation most likely reflects the dendritic stunting and degeneration of pyramidal cells in DAT. This hippocampal region exhibits the highest density of neuritic plaques, tangles and granulovacuolar degeneration in DAT [1, 7, 9]. The selective loss of $GABA_B$ receptors in the dentate gyrus of DAT hippocampus may reflect the observed pathology in this region. Hyman et al. [10] have noted neuritic plaque formation in the molecular layer of dentate gyrus from DAT hippocampus. These plaques may involve dystrophic axons from entorhinal cortical neurons and/or degenerating dendrites of dentate granule cells [16]. The significant decline in $GABA_B$ receptors in this region may be related to the presence of plaques in this layer. $GABA_B$ receptors in the dentate molecular layer could exist on axon terminals from entorhinal cortical neurons or on dendrites of the granule cells themselves. It is not entirely clear whether the losses in $GABA_B$ receptors in DAT reflect neurodegeneration of entorhinal cortical or dentate granule cells. Studies in the rat suggest the latter case to be true. $GABA_B$ receptors in dentate molecular layer are selectively spared after entorhinal cortical knife-cut lesions, but are lost after colchicine-induced neurotoxicity of granule cells (Chu et al., in preparation). The decrements in GABA receptors we have observed may thus reflect and possibly contribute to the pathological findings and clinical picture of memory impairment associated with DAT.

We wish to thank Genell Fries and Anne Haeckl for excellent secretarial assistance. This work was supported by USPHS Grants AG 06155, NS 15655 and 5 T32 GM07863 (to D.C.M.C.).

1 Ball, M.J., Topographic distribution of neurofibrillary tangles and granulovacuolar degeneration in hippocampal cortex of aging and demented patients. A quantitative study, Acta Neuropathol., 42 (1978) 73–80.

2 Ben-Ari, Y., Krnjevic, K., Reiffenstein, R.J. and Reinhardt, W., Inhibitory conductance changes and action of gamma-aminobutyrate in rat hippocampus, Neuroscience, 6 (1981) 2445–2463.

3 Bowery, N.G., Classification of GABA receptors. In S.J. Enna (Ed.), The GABA receptors, Humana, Clifton, N.J., 1983, pp. 177–213.

4 Chu, D.C.M., Penney, J.B. and Young, A.B., Cortical $GABA_B$ and $GABA_A$ receptors in Alzheimer's disease: a quantitative autoradiographic analysis, Neurology, 37 (1987) 1454–1459.

5 Gahwiler, B.H. and Brown, D.A., $GABA_B$-receptor-activated $K^+$ current in voltage-clamped $CA_3$ pyramidal cells in hippocampal cultures, Proc. Natl. Acad. Sci. USA, 82 (1985) 1558-1562.

6 Gamrani, H., Onteniente, B., Seguela, P., Geffard, M. and Calas, A., $\gamma$-Aminobutyric acid-immunoreactivity in the rat hippocampus. A light and electron microscopic study with anti-GABA antibodies, Brain Res., 364 (1986) 30–38.

7 Gibson, P.H., Form and distribution of senile plaques seen in silver impregnated sections in the brain s of intellectually normal elderly people and people with Alzheimer-type dementia, Neuropathol. Appl. Neurobiol., 9 (1983) 379–389.

8 Hardy, J., Cowburn, R., Barton, A., Reynolds, G., Dodd, P., Wester, P., O'Carroll, A., Lofdahl, E. and Winblad, B., A disorder of cortical GABAergic innervation in Alzheimer's disease, Neurosci. Lett., 73 (1987) 192–196.

9 Hyman, B.T., Van Hoesen, G.W., Damasio, A.R. and Barnes, C.L., Alzheimer's disease: cell-specific pathology isolates the hippocampal formation, Science, 225 (1984) 1168–1170.

10 Hyman, B.T., Van Hoesen, G.W., Kromer, L.J. and Damasio, A.R., Perforant pathway changes and the memory impairment of Alzheimer's disease, Ann. Neurol., 20 (1986) 472–481.
11 Khachaturian, Z.S., Diagnosis of Alzheimer's disease, Arch. Neurol., 42 (1985) 1097–1105.
12 Manchon, M., Kopp, N., Bobillier, P. and Miachon, S., Autoradiographic and quantitative study of benzodiazepine-binding sites in human hippocampus, Neurosci. Lett., 62 (1985) 25–30.
13 Newberry, N.R. and Nicoll, R.A., Comparison of the action of baclofen with γ-aminobutyric acid on rat hippocampal pyramidal cells in vitro, J. Physiol., 360 (1985) 161–185.
14 Ottersen, O.P. and Storm-Mathisen, J., Neurons containing or accumulating transmitter amino acids. In A. Bjorklund, T. Hokfelt and M.J. Kuhar (Eds.), Handbook of Chemical Neuroanatomy, Vol. 3, Classical Transmitters and Transmitter Receptors in the CNS, Part II, Elsevier, 1984, pp. 141–246.
15 Pan, H.S., Frey, K.A., Young, A.B. and Penney, J.B., Changes in [$^3$H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative autoradiography, J. Neurosci., 3 (1983) 1189–1198.
16 Probst, A., Basler, V., Bron, B. and Ulrich, J., Neuritic plaques in senile dementia of Alzheimer type: a Golgi analysis in the hippocampal region, Brain Res., 268 (1983) 249–254.
17 Reubi, J.C., Cortés, R., Maurer, R., Probst, A. and Palacios, J.M., Distribution of somatostatin receptors in the human brain: an autoradiographic study, Neuroscience, 18 (1986) 329–346.
18 Rossor, M.N., Emson, P.C., Mountjoy, C.Q., Roth, M. and Iversen, L.L., Reduced amounts of immunoreactive somatostatin in the temporal cortex in senile dementia of Alzheimer type, Neurosci. Lett., 20 (1980) 373–377.
19 Schmechel, D.E., Vickrey, B.G., Fitzpatrick, D. and Elde, R.P., GABAergic neurons of mammalian cerebral cortex: widespread subclass defined by somatostatin content, Neurosci. Lett., 47 (1984) 227 232.
20 Scoville, W.B. and Milner, B., Loss of recent memory after bilateral hippocampal lesions, J. Neurol. Neurosurg. Psychiat., 20 (1957) 11–21.
21 Storm-Mathisen, J. and Ottersen, O.P., Neurotransmitters in the hippocampal formation. In F. Reinoso-Suarez and C. Ajmone-Marsen (Eds.), Cortical Integration, Raven, New York, 1984, pp. 105 130.
22 Walker, L.C., Kitt, C.A., Struble, R.G., Schmechel, D.E., Oertel, W.H., Cork, L.C. and Price, D.L., Glutamic acid decarboxylase-like immunoreactive neurites in senile plaques, Neurosci. Lett., 59 (1985) 165-169.
23 Zimmer, R., Teelken, A.W., Trieling, W.R., Weber, W., Weihmayr, T. and Lanter, H., γ-Aminobutyric acid and homovanillic acid concentration in the CSF of patients with senile dementia of Alzheimer's type, Arch. Neurol., 41 (1984) 602–604.