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Histological characterization of interneurons in Alzheimer's Disease reveals a loss of somatostatin interneurons in the temporal cortex

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Abstract

Neuronal dysfunction and synaptic loss are major hallmarks of Alzheimer's Disease (AD) which correlate with symptom severity. Impairment of the GABAergic inhibitory interneurons, which form around 20% of the total neuronal network, may be an early event contributing to neuronal circuit dysfunction in neurodegenerative diseases. This study examined the expression of two of the main classes of inhibitory interneuron, parvalbumin (PV) and somatostatin (SST) interneurons in the temporal cortex and hippocampus of AD and control cases, using immunohistochemistry. We report significant regional variation in the number of PV and SST interneurons with a higher number identified per mm^2 in the temporal cortex compared to the hippocampus. Fewer SST^+ interneurons, but not PV^+ interneurons, were identified per mm^2 in the temporal cortex of AD cases compared to control subjects. Our results support regional neuroanatomical effects on selective interneuron classes in AD, and suggest that impairment of the interneuronal circuit may contribute to neuronal dysfunction and cognitive decline in AD.

Keywords

Alzheimer disease, Interneurons, Pathology, Humans, Somatostatin

Introduction

Neuronal dysfunction and synaptic loss are key features of Alzheimer's Disease (AD) which correlate with symptom severity [1]. Pyramidal neurons have been the main focus of study in relation to AD pathology [2], with cholinesterase inhibitors commonly used for symptomatic relief in AD. However, interneurons are also essential to the normal functioning of the neuronal network. Forming around 20% of the total neuronal network [3], most interneurons are γ-aminobutyric acid (GABA)ergic and inhibitory, also acting as a source of neuropeptides that help to regulate cortical function [4].

There are three main classes of inhibitory interneuron with calcium-binding proteins and neuropeptides used to distinguish between them. The different types of interneuron provide GABA, a major inhibitory neurotransmitter, to different subcellular places with the most common class of cortical interneurons, being the calcium-binding protein parvalbumin (PV) expressing interneurons. These cells comprise of the chandelier cells which innervate the axon initial segment, basket cells that target the soma of excitatory cells, and trans-laminar interneurons [5]. These interneurons are responsible for their fast-spiking activity and precise inhibitory control of pyramidal cell output [6]. Somatostatin (SST) expressing interneurons, the Martinotti and non-Martinotti cells, fire more randomly and gradually to stimulus targeting dendrites of excitatory neurons [7]. SST^+ cells release GABA that acts on a wide array of GABA receptors (GABAR), alongside fast synaptic $GABA_AR$, the slower more persistent metabotropic $GABA_BRs$ become activated on pyramidal cells silencing connections between these excitatory cells [8]. Furthermore it has been proposed that the GABA released from SST cells affects the connectivity between excitatory neurons, regardless of whether these excitatory neurons are synaptically connected with SST neurons [9].

The third class of interneurons express the serotonin receptor 5HT3aR as well as expressing vasoactive intestinal peptide (VIP) and are distinguishable for synapsing PV^+ and SST^+ interneurons [10].Typical VIP expressing interneurons are bipolar displaying continuous adapting firing properties [11].

Cognitive symptoms associated with AD are the result of altered neural networks including abnormal oscillatory rhythmic activity and network hypersynchrony that can occur many years prior to clinical symptom onset [12-14]. Studies have shown the involvement of interneurons in regulating these neural circuits and networks to be altered in AD, suggesting that interneuron dysfunction could play a role in neuronal network failure and cognitive dysfunction in AD [15]. The Tg8CRND8 mouse model of AD exhibits spatial memory deficits and altered anxiety alongside a reduced number of interneurons. Treatment with α-

melanocyte stimulating hormone (α-MSH) improved spatial memory and prevented the loss of SST^+ interneurons, demonstrating its role in restoring GABAergic inhibition to improve cognition in this model [16]. Similarly, in the APP/PS1 mouse model of AD SST^+ interneurons decrease with aging compared to control mice, while PV^+ interneurons were increased suggesting a diverse interneuron population vulnerability that may be related to amyloid beta (Aβ) pathology in AD [17].

Previous work has shown that the enzymes responsible for degrading Aβ are expressed in interneurons [18]. The presence of endothelin-converting enzyme (ECE-2) and neprilysin in interneurons suggests the possibility that Aβ may have a role in the regulation of inhibition, acting as a neuropeptide important for interneuronal function [18]. Additionally, γ-aminobutyric acid (GABA) receptors are altered in AD [19] with a varied pattern of $GABA_B$ receptor R1 protein (GBR1) expression throughout the hippocampus of AD patients. An increased GBR1 expression was identified in the CA4 and CA3/2 areas, yet was rapidly reduced in the CA1 region with advanced AD pathology and progression of neurofibrillary tangles (NFT) prior to neuronal cell death [19]. These GABA receptors have been shown to be involved in the inhibitory neurotransmission system that could contribute to neuronal resistance seen in the initial stages of disease pathology. Thus changes in the balance between inhibitory and excitatory neurotransmission are likely to contribute to AD development. The learning and memory deficits that correlated with the age-dependent decline in SST^+ interneurons in the apoE4-KI mouse model of AD were rescued following the restoration of GABA signaling using pentobarbital, a $GABA_A$ receptor enhancer [5]. Another mutated amyloid precursor protein (APP) familial mouse model of AD exhibited memory deficits and a reduction in GABA related proteins and GABAergic interneurons as early as 4 months [20]. With APP known to play a role in GABAergic synaptic formations, by administrating diazepam and correcting the APP function an improvement in memory and also reduced Aβ accumulation was seen. Clearly, the GABAergic deficiency caused memory deficits and contributed to Aβ accumulation in this model [20].

We have investigated changes in two of the main subclasses of inhibitory interneurons, the PV- and SST- expressing interneuron in temporal structures of AD and age-matched control brain using an immunohistochemistry approach. We hypothesized that inhibitory neurons are lost in areas of the brain typically affected by pathology in AD.

Materials and Methods

Human central nervous system tissue

All formalin fixed paraffin embedded (FFPE) lateral temporal cortex (Brodmann areas 21/22) and hippocampus tissue was obtained from the Sheffield Brain Tissue Bank (SBTB). The SBTB gave full ethical approval for the use of tissue in this study (ref. 08/MRE00/103+5) as a Research Tissue Bank approved by the Scotland Research Ethics Committee. A summary of the cohort used in the study is provided in Table 1.

Immunohistochemistry

Immunohistochemistry (IHC) was performed using a standard avidin-biotin complex-horse radish peroxidase (ABC-HRP) method, and visualized with diaminobenzidine (DAB) (Vector Laboratories, UK). Briefly, deparaffinised 5µm sections were rehydrated and endogenous peroxidase quenched by blocking the sections in 3% H_2O_2/methanol for 20 minutes at room temperature (RT). Following antigen retrieval (Table 2) sections were blocked in 1.5% normal serum for 30 minutes at RT. Sections were incubated in the relevant optimal antibody dilution, washed thoroughly in tris buffered saline (TBS, pH7.5) and incubated in 0.5% biotinylated secondary antibody for 30 minutes at RT. After thorough washing in TBS the sections were incubated in ABC-HRP for 30 minutes at RT before a final wash in TBS and incubation in the substrate DAB for 5 minutes at RT, sections were dehydrated, cleared in xylene and mounted for image analysis. Negative controls (omission of the primary antibody and isotype controls) were incubated in each run. Additional dual labelling was completed where SST stained sections (using a standard ABC-HRP method as earlier) were incubated with the avidin-biotin blocking kit (Vector Laboratories, UK), and incubated overnight at 4°C with anti-PV, followed by the alkaline-phosphatase-conjugated ABC (Vectastain Elite kit, Vector Laboratories, UK), developed with alkaline phosphatase substrate (Vector Laboratories, UK; red) and counterstained with haematoxylin. Negative controls consisted of sections incubated in the absence of primary antibody.

Quantification of antibody staining and statistical analysis

Immunostained sections were imaged using either a Nikon Eclipse 80i microscope (Nikon UK, Kingston upon Thames) or digitally scanned under a 40x objective lens using a Nanozoomer XR (Hamamatsu, Photonics Ltd., Hertfordshire, UK). Scanned sections were stored as NanoZoomer Digital Pathology Image (.ndpi) files, viewed and exported using NDP.View 2.

Quantification of PV-specific immunoreactivity within the temporal cortex was performed by capturing non-overlapping bright-field microscope images at x20 magnification in three contiguous belt transects covering the total cortical thickness (Supp Fig 1). Within the hippocampus, five non-contiguous images along the pyramidal layer were exported from the

scanned sections at x20 magnification in the CA1 region, and three images in the subiculum. Non-overlapping images were exported from the scanned sections at x20 magnification across the thickest region of the entorhinal cortex in two contiguous belt transects (Supp Fig 2). PV immunopositive cells were manually counted in each region as well as the total percentage PV area immunoreactivity analysed in Analysis^D, (Olympus Biosystems, Watford, UK).

SST^+ cells were more infrequent than PV, and therefore quantification was performed solely by manual cell counts rather than using computer-aided image analysis. Within the temporal cortex, SST immunopositive cells were counted in the area of highest expression in six non-overlapping images exported from the scanned sections at x20 magnification in a contiguous belt. Within the CA1 and enthorinal cortex the areas of highest expression within the hippocampus, SST immunopositive cells were counted in six random images exported from the scanned sections at x20 magnification in both the CA1 and entorhinal cortex regions.

All statistical analyses were performed using IBM SPSS Statistic v24. For variation between neuroanatomical regions, Friedman's Two-Way Analysis of Variance was used to compare immunoreactivity in the four brain regions (temporal cortex, hippocampus CA1, subiculum and entorhinal cortex) across the full cohort. Post hoc differences were assessed by Wilcoxon Signed Rank Test. Statistical comparisons of quantitative data between the control and AD cases was performed using Mann-Whitney U Tests.

Results

Somatostatin (SST) interneurons were reduced in the temporal cortex of AD patients

SST immunoreactivity was discretely associated with the cytoplasm of neuronal cell bodies and immediate extending dendrites (Figures 1a&b), therefore for quantification total cell counts were used (Table 3). In addition, immunoreactivity was located predominantly at the grey matter/white matter border (Figures 1c&d). There was significant neuroanatomical regional variation in the number of SST^+ interneurons ($F=18.96$ 2df $p=<0.001$) with an increased number in the temporal cortex compared to CA1 ($p=0.001$) and entorhinal cortex ($p=0.006$). There was a slight increase in the number of SST^+ cells in the entorhinal cortex compared to CA1 regions ($p=0.025$) (Figure 2a). Overall, there was a reduction (of approximately 30%) in the number of SST^+ interneurons in the temporal cortex of AD patients compared to control cases (Mann-Whitney U test $p=0.040$), although this did not achieve statistical significance if corrected for multiple testing using the Bonferroni method ($p=0.102$). No significant differences in the number of SST^+ interneurons were detected between the

entorhinal cortex or CA1 regions of the hippocampus of AD patients and control cases (Mann-Whitney U test p=0.382, p=0.673 respectively (Figure 2b).

Parvalbumin (PV) immunoreactivity of neuronal cell bodies was more pronounced in a band like pattern of the outer layers of the cortex

PV immunoreactivity was detected in the cytoplasm of neuronal cell bodies and immediate extending dendrites (Figures 3a&b). For quantification both the total percentage area of PV immunoreactivity and total cell count were used (Table 3). Immunoreactivity appeared higher in the more outer cortical layers (I-IV) of the temporal and entorhinal cortex in a band like pattern (Figure 3c). Within the hippocampus the staining pattern varied greatly with PV immunoreactivity of cell bodies in CA1 being more sparsely distributed than in the temporal cortex (Figure 3d), while in the subiculum the immunoreactivity appeared in clusters (Figure 3e). One of the AD cases showed very limited cytoplasmic cell body staining with immunoreactivity restricted to the surface of neurons in a beaded string like manner, suggesting a synaptic bouton labelling pattern (Figure 3f).

There was a significant difference in PV immunoreactive area (F= 37.87 3df p=<0.001) and cell count (F= 32.50 3df p=<0.001) across all four brain regions investigated. The temporal cortex had the highest total PV immunoreactivity and cell count per mm^2 compared to the other three areas of the hippocampus (CA1, subiculum, entorhinal cortex) (p=<0.001). Within the hippocampus PV immunoreactivity was significantly higher in the subiculum compared to CA1 (p=<0.001) and entorhinal cortex (p=0.001) (Figure 4a). There was no significant difference in PV immunoreactivity between the CA1 and entorhinal cortex (p=0.485) (Figure 4a).

For cell count, the temporal cortex had significantly more PV immunopositive neurons per mm^2 compared to the CA1 and entorhinal cortex (p=<0.001 (Figure 4b). In contrast, there was no significant difference in the number of PV positive neurons per mm^2 in the subiculum compared to the temporal cortex (p=0.372), likely reflecting extensive case to case variation in the immunoreactive profile (Figure 4b), Within the hippocampus the subiculum contained significantly more PV immunoreactive cells per mm^2 than CA1 (p=<0.001) and entorhinal regions (p=0.004), with an increase number of PV positive cells per mm^2 in the entorhinal regions compared to CA1 (p=0.044) (Figure 4b).

Parvalbumin (PV) immunoreactivity was not altered in AD and did not colocalise with somatostatin (SST) immunoreactivity

There were no significant differences in total PV immunoreactive area or cell count per mm^2 between AD and control cases regardless of the brain region investigated (Supp Fig 3). Additionally no colocalisation of PV and SST immunoreactivity was present (Supp Fig 4).

Discussion

This study suggests a trend to a decrease in SST^+ interneurons per mm^2 in the temporal cortex of AD cases whilst, in contrast, no significant difference in PV^+ interneurons per mm^2 was identified in AD cases compared to control subjects, in several brain area investigated. Overall there are significant differences in the number of PV^+ and SST^+ interneurons per mm^2 across neocortical and hippocampal subregions within the temporal lobe, with more of both types of interneuron identified in the temporal cortex compared to hippocampal subfields.

A major role of interneurons is to influence neuronal circuits by modulating the action of excitatory neurons. SST and PV expressing cells are subsets of GABAergic interneurons [9], each providing GABAergic input to specific subcellular domains at defined rates and times [6]. The unique entity of each interneuron is shown by the lack of colocalisation between PV and SST expressing interneuron which is supported by other work identifying the non-overlapping groups of interneurons [21-24]. Alterations in the gene expression, neural activity and anatomy of SST^+ interneurons have been identified in a number of psychiatric and neurological disease including schizophrenia, seizure disorders and epilepsy [9]. In particular SST depletion in the cortex and hippocampus of AD patients has been connected to memory and learning impairment [25, 26]. Similarly, SST interneuron decline has been identified in a number of AD animal models strongly correlating with memory and learning impairments [16, 27-29]. By restoring GABA receptor signalling with pentobarbitol following GABAergic interneuronal loss, memory and learning deficits were rescued in the apoE4-KI mice [5]. Also, the use of the neuroprotective peptide α-MSH attenuated GABAergic interneuron loss and improved cognition in the TgCRND8 mouse model of AD [16].

Repetitive activity in pyramidal neurons can drive SST^+ interneurons into providing feedback inhibition [30, 31]. Consequently, a decrease in SST^+ interneurons in AD temporal cortex as seen in the current study, could act as a protective measure, enabling the continuing excitation of surviving pyramidal neurons to compensate for their progressive loss seen in the disease.

Sub-regions of the temporal cortex have important roles in coordinating hippocampal functions, therefore the reduction in SST^+ interneurons within the temporal cortex of AD patients could suggest a disruption in the overall cortico-hippocampal network and loss of

inhibition downstream causing over excitation of pyramidal neurons in the hippocampus [32]. Ultimately this could lead to associated negative effects such as an increase in oxidative stress, DNA damage and dysregulation of intracellular calcium that could contribute to neuronal death associated with AD [33].

Previous work has shown several hundred somatostatin labelled neurons in layer II/III of the temporal cortex [34] however, this work investigated three epilepsy cases aged 25-30 yrs, thus differing in both age and pathology of the subjects in the current study. SST decrease in the brain with increasing age [35]; this is further heightened in AD [36-39]. Remaining SST^+ neuronal processes in AD are located in close proximity to neuritic plaques in the cingulate, frontal, temporal cortex [40] and hippocampus [41]. An early reduction in SST^+ interneurons in the olfactory cortex of an AβPP/PS1 double transgenic mouse model of AD [37] has since been shown in human AD post mortem tissue where SST also colocalised with amyloid-beta (Aβ) in the olfactory cortex [38]. In contrast SST^+ interneurons rarely colocalised with tau protein [38]. In AD the accumulation of Aβ has been suggested to be caused by the impaired clearance of the protein [42]. It may be speculated that a loss of SST^+ interneurons in AD, as seen in this study and others, may lead to a loss in Aβ degrading enzymes, including endothelin-converting enzyme ECE-2 and therefore reduced Aβ metabolism and clearance [18] resulting in Aβ accumulation and induced cell death [43]. Through mass spectrometry studies the most pronounced peptide to bind to Aβ was the cyclic neuroendocrine peptide somatostatin-14 (SST-14) [44] highlighting the likely importance of the role of SST interaction with Aβ surrounding AD pathology.

There is conflicting literature as to the significance of PV^+ interneurons in AD. Human post mortem brain studies have shown a loss of PV^+ interneurons in areas known to be affected early in the disease, including the entorhinal cortex and hippocampus [45-47] as well as in the hippocampus of patients with dementia with Lewy bodies [48]. However, in contrast, other human studies showed little variation in PV^+ interneurons in AD subjects, similar to our current findings, suggesting PV^+ interneurons are resistant to degeneration in AD [49-51].

However, despite no changes, PV-expressing synaptic boutons were identified surrounding pyramidal neurons in the hippocampus of some AD patients. This could suggest that as neurons are lost in the hippocampus, an area involved in early AD pathology, an increasing lack of stimulus onto GABAergic interneurons occurs. Ultimately this lack of innervation could lead to PV translocating to axonal terminals to maintain calcium homeostasis and synaptic inhibition in the remaining pyramidal neurons, as the dysregulation of intracellular calcium homeostasis due to synaptic impairment has been previously identified as an initiating factor in AD [52]. However, a further detailed quantification of these synaptic boutons

is required in a larger sample size to confirm this. Therefore the overall loss of PV^+ interneurons may be delayed and not seen until later in the disease as suggested in an AβPP/PS1 double transgenic mouse model of AD [37] where the differential vulnerability among interneuron populations was possibly related to Aβ pathology [17]. Parvalbumin immunoreactivity in the subiculum was localised to the parvopyramdial clusters in the current study which has previously shown to be areas immunopositive for depositions of amyloid-Bri, an amyloidogenic fragment associated with a stop codon mutation in the *BRI* gene [53]. However, no difference in parvalbumin immunoreactivity in the parvopyramdial clusters was identified between controls and AD subjects in this study possibly reflecting the small sample size.

Alternatively, neuronal loss in AD and resulting loss of excitable input may cause interneuron dysfunction, rather than degeneration, which could explain the translocation of PV to synaptic boutons [54, 55]. This translocation of PV could cause detrimental changes in the protein's function causing calcium dysregulation and impaired interneuron inhibition, increasing pyramidal neuron excitability and ultimately neuronal loss associated with the disease. Restoring the function of PV^+ interneurons has been shown to restore inhibitory synaptic transmission, network activity and cognitive deficits in human amyloid precursor protein (hAPP) transgenic mice [56].

Development of effective therapeutics for treating AD will come about through a better understanding of the mechanisms underlying neuronal dysfunction and loss in the disease. Our current findings, the first neuropathological study investigating PV and SST interneuron distribution throughout the temporal cortex and hippocampus of human AD patients compared to control subjects suggest interneuron changes in AD may be selective to specific interneuron populations and anatomical location. However, these conclusions are based on investigations carried out on only a small number of cases and two subclasses of interneuron. In order to better understand the significance and of interneurons in AD, a much larger study examining more cases and investigating the various interneuron populations across different anatomical regions in the human AD brain is warranted.

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Figure legends

Figure 1 Somatostatin immunoreactivity. Somatostatin immunoreactivity was associated with the cytoplasm of interneuronal cell bodies (**a**, black arrows) and immediate dendritic processes (**b**, blue arrows) throughout the temporal cortex and hippocampus. Immunoreactivity was primarily located at the grey matter / white matter border (**c**, low magnification, **d** high magnification). *Scale bar* **a** 100µm, **b** 100µm, **c** 500µm, **d** 100µm.

Figure 2 Quantification of somatostatin immunoreactivity. There was significantly higher number of somatostatin immunoreactive interneurons per mm^2 in the temporal cortex compared to CA1 and entorhinal cortex across all cases (AD and controls) investigated in this study (**a**). There was a reduction in the number of somatostatin interneurons per mm^2 in the temporal cortex of AD patients compared to control cases (**b**). No differences were seen in the number of somatostatin interneurons per mm^2 between AD and control cases in the other areas investigated (CA1 and entorhinal cortex) (**b**). *P=<0.05, **P=<0.01 ***P=<0.001.

Figure 3 Parvalbumin immunoreactivity. Temporal and entorhinal cortex showed a regular pattern of parvalbumin positive interneurons with cytoplasmic (black arrow) and dendritic immunoreactivity (blue arrow) (**a** lower magnification, **b** higher magnification). This pattern of staining appeared in a band like pattern concentrating in the outer layers (I-IV) of the cortex (**c**). Within the CA1 region the immunoreactivity was more dispersed with cytoplasmic (black arrow) and dendritic immunoreactivity (blue arrow) (**d**) and immunoreactivity in the subiculum appeared in clusters (**e** black arrows). A bead-string like pattern of immunoreactivity was present lining neuronal cell bodies of areas of AD hippocampus (circled) (**f**). *Scale bar* **a** 250µm, **b** 100µm, **c** 500µm, **d** 100µm, **e** 500µm, **f** 50µm.

Figure 4 Quantification of parvalbumin immunoreactivity. Parvalbumin positive cells were counted and expressed as the total number of positive cells per mm^2 (**a**). There overall total percentage parvalbumin immunoreactivity was calculated per total area examined (**b**). *P=<0.05, **P=<0.01 ***P=<0.001.

Supplementary Figure 1. An illustration of transect belt sampling in the temporal cortex. Non-overlapping images were taken across the cortical layers at X20 magnification in three contiguous belts. The number of images in each belt varied according to the thickness of the cortex.

Supplementary Figure 2. An illustration of plot sampling and transect belt sampling in the hippocampus. Five randomly distributed images were taken from the CA1 region (blue)

and three randomly distributed images from the subiculum (red). Non-overlapping images were taken across the entorhinal cortex in two contiguous belts (green).

Supplementary Figure 3. Boxplots showing total parvalbumin percentage area in the temporal cortex (**a**), subiculum (**b**), CA1 (**c**), and entorhinal cortex (**d**) and total cell count in the temporal cortex (**e**), subiculum (**f**), CA1 (**g**), and entorhinal cortex (**h**) in Alzheimer's disease compared to control cases.

Supplementary Figure 4. Somatostatin and parvalbumin interneurons are distinct cells. (a) Dual immunoreactivity of somatostatin interneurons (brown cells, blue arrows) showed no colocalisation with parvalbumin interneurons (red cells, black arrows) lower magnification. (b) Parvalbumin$^+$ interneurons, higher magnification, (c,d) somatostatin$^+$ interneuron, higher magnification.

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ID	Median age (years) (min-max)	Sex (F/M)	PMD (hrs)*	Cause of death (based on clinical information)
Control (9 hippocampus blocks, 9 temporal cortex blocks)	74 (59-84)	(5/4)	47 (5-75)	1. No neurological disease. Brain age-related changes. 2. Atypical pneumonia. Brain no abnormality. 3. Sudden death, history of epilepsy. Brain no abnormality 4. Guillan-Barre Syndrome, metastatic carcinosarcoma. Brain lacunar infarct. 5. Subarachnoid haemorrhage. No neurodegenerative pathology. 6. Pneumonia, carcinoma of the bladder. Brain - basal Ganglia calcification. 7. Cardiac failure, liver failure, chronic hepatitis C. Brain age-related changes. 8. Hepatocellular carcinoma, cirrhosis. Brain no significant abnormality. 9. Renal failure. Brain - no significant abnormality.
AD (9 hippocampus blocks, 9 temporal cortex blocks)	75 (59-93)	(5/4)	62 (24-96)	Alzheimer's Disease Braak Stage 5 (n=4), Braak Stage 6 (n=5). Neuritic plaque staging according to CERAD, moderate neuritic plaques (n=3), severe neuritic plaques (n=6).

317 **Table 1.** Age, sex, post mortem delay (PMD) and cause of death of SBTB brain donors.

318 *Information not available for 10 individuals (6 controls, 4 AD)

319 Key: SBTB, Sheffield Brain Tissue Bank; F, female; M, male

Antibody	Specificity	Isotype	Dilution (time, Temp)	Antigen retrieval method	Supplier
Parvalbumin	Ca^{2+} binding protein	Rabbit IgG	1:500 (1hr, RT)	MW, 10 mins, TSC	Abcam (ab11427)
Somatostatin	neuropeptide	Rabbit IgG	1:100 (1hr, RT)	MW, 10 mins, EDTA	Abcam (ab108456)

Table 2. Antibody source and specificity

Key: RT, room temperature; MW, microwave; TSC, trisodium citrate buffer pH 6.5; EDTA, Ethylenediamine Tetra-acetic Acid pH 8.0.

	Parvalbumin				Somatostatin	
	Number of positive cells per mm^2		Total percentage immunoreactivity		Number of positive cells per mm^2	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
Temporal cortex						
Control	29.68 (6.85)	28.04 (11.92)	8.87 (4.54)	7.26 (8.42)	2.07 (0.63)	2.05 (0.82)
AD	26.41 (9.19)	21.84 (8.71)	9.30 (3.35)	8.18 (2.35)	1.49 (0.51)	1.43 (0.41)
CA1						
Control	4.58 (5.40)	3.77 (4.72)	0.15 (0.20)	0.05 (0.19)	0.49 (0.60)	0.41 (1.23)
AD	3.63 (4.69)	1.89 (7.55)	0.18 (0.17)	0.14 (0.28)	0.82 (0.37)	0.82 (0.62)
Subiculum						
Control	21.78 (14.53)	17.30 (25.15)	1.80 (2.45)	0.63 (2.01)		
AD	25.15 (17.10)	15.72 (34.60)	0.76 (0.68)	0.44 (1.07)		
Entorhinal cortex						
Control	8.87 (3.53)	9.14 (4.32)	0.14 (0.08)	0.13 (0.14)	1.14 (0.56)	1.23 (1.23)
AD	9.63 (8.00)	15.09 (14.99)	0.23 (0.35)	0.12 (0.24)	1.05 (0.83)	0.82 (1.03)

Table 3. Quantification of Parvalbumin and Somatostatin staining

Key: SD, standard deviation; IQR, inter-quartile range

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