Articles

Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease

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Summary

The distinction between the neurodegenerative changes that accompany normal ageing and those that characterise Alzheimer's disease is not clear. The resolution of this issue has important implications for the design of therapeutic and investigative strategies. To this end we have used modern stereological techniques to compare the regional pattern of neuronal cell loss in the hippocampus related to normal ageing to that associated with Alzheimer's disease.

The loss related to normal ageing was evaluated from estimates of the total number of neurons in each of the major hippocampal subdivisions of 45 normal ageing subjects who ranged in age from 13 to 101 years. The Alzheimer's disease related losses were evaluated from similar data obtained from 7 cases of Alzheimer's disease and 14 age matched controls. Qualitative differences were observed in the regional patterns of neuronal loss related to normal ageing and Alzheimer's disease. The most distinctive Alzheimer's disease related neuron loss was seen in the CA1 region of the hippocampus. In the normal ageing group there was almost no neuron loss in this region (final neuron count in the CA1 region: $4{\cdot}40 \times 10^6$ neurons for the Alzheimer's disease group *vs* $14{\cdot}08 \times 10^6$ neurons in the normal ageing group).

It is concluded that the neurodegenerative processes associated with normal ageing and with Alzheimer's disease are qualitatively different and that Alzheimer's disease is not accelerated by ageing but is a distinct pathological process.

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Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disease of the central nervous system characterised by changes in personality, deterioration of cognitive function, loss of neurons, and presence of senile plaques and neurofibrillary tangles in specific regions of the brain.[1-2] The aetiology of the disease is poorly understood and its relation to degenerative processes associated with normal ageing is a source of debate.[3-4] The increased frequency of the disease with age[5] and the presence of Alzheimer-type pathology in non-demented elderly individuals,[6-9] suggest that the distinction between AD and ageing is quantitative and that the causes and mechanisms are the same.[10] Evidence of rapid atrophy in the medial temporal lobe of AD patients[11] and environmental factors, such as trauma,[12] aluminium,[13] and viral[14] and genetic risk factors such as those related to apolipoprotein E[15,16] and amyloid precursor proteins,[17] suggest that AD involves a separate degenerative process. The issue of whether AD is accelerated ageing or a true disease involving a unique pathological process is important in determining whether investigative and therapeutic strategies should focus on a normal ageing process or on an independent pathological process. We have used stereological techniques to identify differences between the patterns of neurological degeneration related to normal ageing and AD.

Our study focuses on the hippocampal region, located on the baso-medial part of the temporal lobe. This region is one of the first to develop the neuropathological signs of AD and, in advanced cases, is the region most profoundly affected.[18,19] Because the hippocampal region is also involved in aspects of memory processes that deteriorate with AD,[20] we thought that the structure would be altered by the degenerative processes associated with AD and that the region would be an appropriate part of the brain in which to compare degenerative changes related to AD and normal ageing.

The patterns of degeneration were evaluated in terms of the neuronal loss observed in the subdivisions of the hippocampal region. Unlike senile plaques and neurofibrillary tangles, neuronal loss reflects the cumulative tissue damage accrued with age and AD.[21] There are few reliable reports of AD and age related losses[22] because methods for making precise estimates of the total number of neurons in localised areas of the brain were not available when most of these studies were done. Data obtained with recently developed stereological techniques,[23] indicate that there is normal age-related

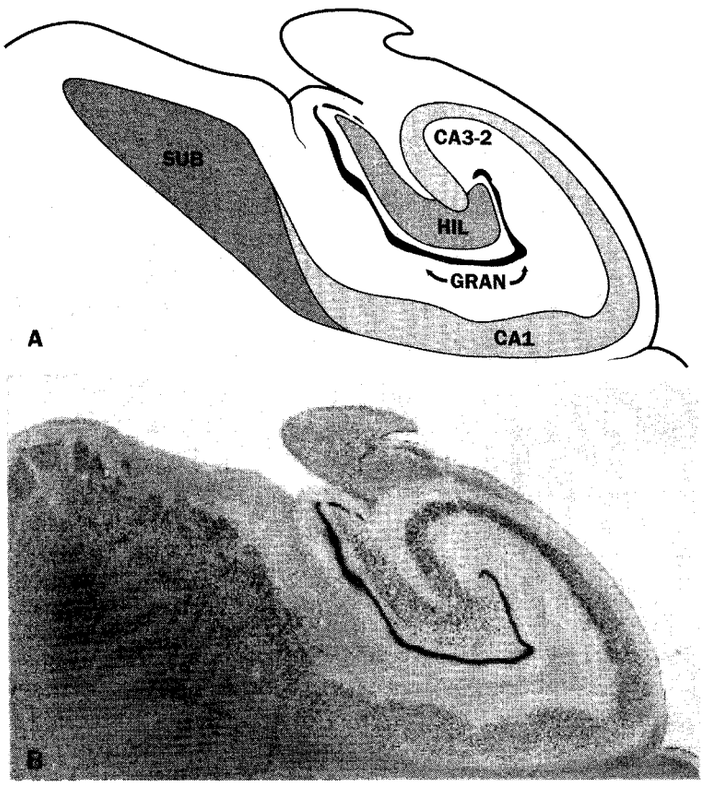

Figure 1: **A: Diagram showing the transverse organisation of the subdivisions of the hippocampal region seen in the histological section in B**

Total neuron number was estimated in GRAN, the dentate granule cell layer; HIL, the dentate hilus; CA3–2 and CA1, the pyramidal neurons of the hippocampus proper; and SUB, the subiculum. B: Histological section through the dorsal-medial part of the temporal lobe of a non-demented 80 year old subject.

neuronal loss in the hippocampal region which is confined to specific subdivisions.[24] We present additional normative data here to provide a baseline for evaluating the neuronal loss associated with AD.

Methods

Estimates were made of the total number of neurons, in each of the major subdivisions of the hippocampal region on one side of the brain, in histological material obtained at necropsy from 45 male subjects.[25] Before the tissue was removed, the brains were fixed in 4% formalin for between 3 months and two years. The tissue samples were divided into two groups: the normal ageing group, which included samples from 38 subjects who had no history of long-term illness, dementia, or neurological disease (mean age 56 years, range 13–101); and the AD group, samples from 7 demented individuals with both clinical[26] and pathological[27] diagnosis of AD (mean age 79 years, range 68–88). A third group, the age-matched control group, was formed from samples of 14 individuals in the normal ageing group (mean age 78 years, range 64–101). These individuals were selected so that the mean age of the age matched control group most closely approximated the mean age of the AD group. Postmortem delays before fixation ranged from 12–36 hours for the normal ageing group and 6–24 hours for the AD group.

Figure 1 shows the subdivisions of the hippocampal region in which the total number of neurons were estimated: the dentate granule cell layer (DEN); the denate hilus (HIL); the pyramidal cell layer of CA3–2; the pyramidal cell layer of CA1; and the cellular layers of the subiculum (SUB). The estimations were done by multiplying the volume of the layers (obtained with point counting techniques), by the density of neurons in the layers (obtained by systematically sampling the layers with optical disectors[25]). We used 12 to 15 sections, taken at equally spaced intervals along the entire length of the hippocampal region for analysis. The sampling schemes were designed so the true inter-individual variance, not the precision of the individual estimates, made the largest contribution to the group variances. Analysis of the material from the AD group was made without investigator knowledge of the group identity of the material being analysed. That of the normal ageing group was done without the investigator knowing the age of the subjects. Data from 32 of the 38 subjects in the normal ageing group have been published previously as part of a study of normal ageing.[24] 8 of the 14 subjects included in the age-matched control group were also part of that study.

Statistical analysis

Normal age-related neuronal loss was evaluated for each subdivision by testing the regression of total neuron number with age, using the data from the individuals in the normal ageing group. Regressions with 2p values less than 5% were defined as statistically significant. For subdivisions in which there were significant negative relationships, neuronal loss was expressed in terms of the ratio of the predicted neuronal number at the oldest and youngest ages represented in the normal ageing group. AD-related neuronal loss was evaluated for each subdivision with unpaired *t*-tests done on the numerical data from subjects in the AD group and the age matched control group.

Results

Figure 2 shows the total neurons in each of the hippocampal subdivisions for every individual. Significant negative regressions were seen between age and neuron number in the hilus and subiculum (table). The numbers of neurons predicted on the basis of regressions was 2113 000 at age 13 and 1321 000 at age 101 for the hilus, and 7512 000 at age 13 and 4344 000 at age 101, for the subiculum. These differences represent losses of 37% of the neurons in the

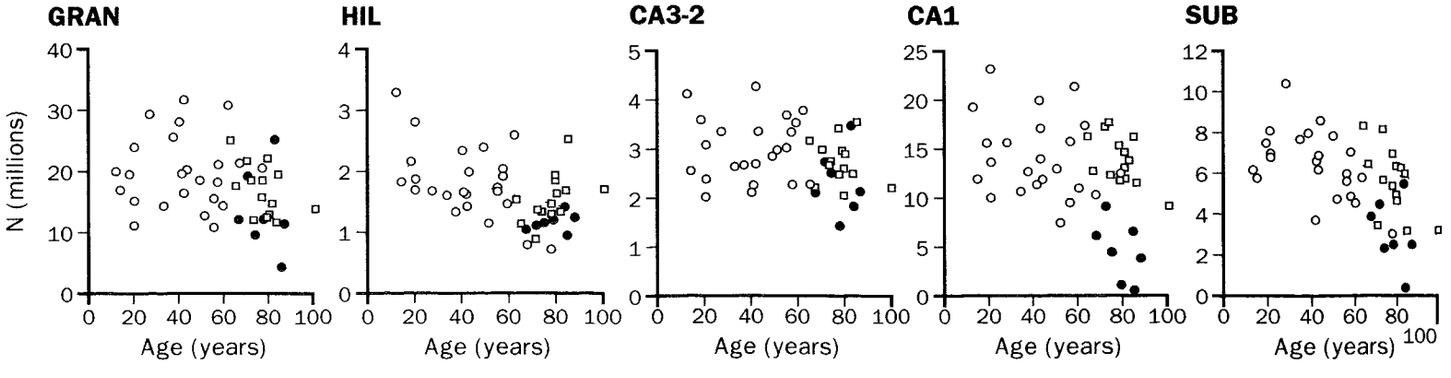

Figure 2: **The total number of neurons (N) in the major subdivisions of the hippocampal regions plotted as a function of age**

Open circles and open squares, the normal ageing group (n = 38); filled circles, the AD group (n = 7); open triangles, the age-matched control group (n = 14).

	Age-related neuronal loss				Alzheimer's related neuronal loss			
	Mean of normal ageing group, neurons × 10^6 (CV) n=38	Slope of neurons vs age (number/year)	2 p of slope	Ageing loss from 13 to 101 years	Mean of age-matched controls neurons × 10^6 (CV) n=14	Mean of AD group neurons × 10^6 (CV) n=7	2 p of AD vs age-matched controls	AD loss
Hippocampal subdivision								
GRAN	18 66 (0 29)	−54 000	0 17		16 84	13 40 (0·50)	0 16	
HIL	1 72 (0 31)	−9000	0 012	37%	1 54 (0 25)	1 16 (0 12)	0 018	25%
CA3–2	2 83 (0 22)	−6000	0 18		2 71 (0 16)	2·28 (0 28)	0 086	
CA1	14 08 (0 26)	−29 000	0 26		13 75 (0 18)	4 40 (0 69)	$3·2-10^{-7}$	68%
SUB	5 95 (0 28)	−36 000	0 0013	43%	5 51 (0 30)	2 93 (0 56)	0 0028	47%

CV = coefficient of variation.

Table: **Analysis of age-related and AD-related neuronal loss in human hippocampus**

hilus and 43% of the neurons in the subiculum over the ages studied.

The mean number of neurons in the hilus, CA1, and the subiculum of the AD group were significantly smaller than those of the age matched control group (table). The most pronounced AD-related reduction in neuron number was observed in CA1, where an average of 68% of neurons were lost and where there was virtually no overlap between the estimates in the individuals in the AD group and those in the age-matched control group. In the subiculum and hilus, the AD-related reductions were 47% and 25%, respectively—beyond those attributed to normal ageing. Although the means of the number of neurons in the dentate granule cell layer and CA3–2 for the AD group were less than those in the respective subdivisions of the age-matched control group, these differences were not statistically significant. Figure 3 shows the percentage of neuron loss because of ageing and AD.

Discussion

Because this study is a longitudinal study with only one measurement per subject, we cannot rule out that the age-related differences are related to secular changes and that differences in the AD and age-matched control groups were present from an early age and do not represent progressive losses of neurons over time. It is possible to argue against the secular interpretation of the normal ageing data, on the basis of the non-heterogeneity of the regional losses, as discussed elsewhere.[24] The longitudinal study of Jobst et al,[11] describing progressive atrophy of the medial lobe of individual AD patients, argues in favour of the interpretation of AD and age-matched control group differences as evidence of neuronal loss. Significant negative regressions for neuron number versus age in the normal ageing group have consequently been discussed in terms of age-related neuronal loss; differences in the means of the numbers of neurons in the AD and age-matched control group in terms of AD-related neuronal loss.

The regional pattern of neuronal loss in the AD group is qualitatively and quantitatively different from that observed in the normal ageing group. We noticed AD-related neuronal loss in two subdivisions that normally lose neurons with age, the hilus and the subiculum, but also noticed neuronal loss in CA1, a region in which there is no evidence of normal age-related loss. The CA1 neuron loss related to AD was greater than that of any other subdivision and there was no overlap between the numbers of CA1 neurons and the AD and aged-control groups. In two individuals from the AD group, CA1 was essentially void of neurons. The loss of neurons in CA1 of the AD group can only be explained by the involvement of a process not involved in normal ageing. It therefore constitutes evidence for a qualitative difference in the regional pattern of neuronal loss in the AD and normal ageing groups and argues against the hypothesis of AD as accelerated ageing.

Our results indicate that AD is not an inevitable consequence of ageing. While this may be comforting, it also implies that the study of normal ageing processes cannot lead to a complete understanding of the degenerative mechanisms involved in AD. AD involves a specific disease process which needs to be understood to provide a basis for specific therapeutic and preventative strategies. Because AD is undeniably linked with age,[28] but not necessarily with the ageing process of the brain, the disease may involve an independent pathological process that proceeds for years or decades before manifesting itself clinically and pathologically. This also suggests that we should search for diagnostic indices long before the clinical signs of AD appear.[29] Although the disease process may require years to become manifest, the actual atrophic phase appears to proceed relatively rapidly.[11] The AD-related neuronal loss described here is most likely associated with this phase.

Neuronal loss with ageing and AD

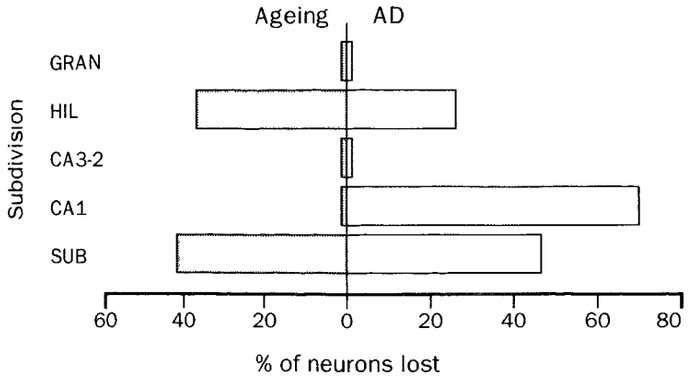

Figure 3: **Percentage of neurons lost due to ageing (left) and to AD (right)**

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This work is dedicated to the memory of Paul B West who died of Alzheimer's disease in 1987.

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