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TOTAL NUMBER OF HIPPOCAMPAL NEURONS IN AIDS PATIENTS AND CONTROLS

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ABSTRACT

The hippocampal region of the brain, an essential component of learning and memory processes, is severely affected in Alzheimer's dementia. Acquired immunodeficiency syndrome (AIDS) is often accompanied by symptoms of dementia characterized by progressive cognitive impairment, personality changes and motor disturbances. A loss of 30% of the neocortical neurons in brains from AIDS patients has been demonstrated in several studies, and this neuron loss appears to be independent of whether or not the patients were clinically demented. With the neocortical loss in mind, the aim of the present study was to investigate a possible neuron loss in the hippocampus in non-demented cases. A stereological method - the optical fractionator - was used to estimate the total number of neurons in the five subregions of the hippocampus in nine AIDS patients and 10 controls. No difference in total neuron number was found between the two groups.

Key words: dementia, hippocampus, stereology, total number of neurons.

INTRODUCTION

The hippocampal region of the brain is an essential component of learning and memory processes (Eichenbaum et al., 1992). The hippocampus is seriously affected in Alzheimer's dementia (AD) (van Hoesen and Hyman, 1990), and a specific loss of neurons in different hippocampal subfields has been demonstrated in severe AD (West et al., 1994; Simic et al., 1997).

The acquired immunodeficiency syndrome (AIDS) is frequently accompanied by disturbances in the central nervous system (CNS). In neuropathological studies abnormalities in CNS in up to 90% of the brains have been described (Navia et al., 1986; Wiley, 1994). One of the major complications is the AIDS dementia complex or HIV-1 associated cognitive/motor complex characterized by disturbances in cognitive, behavioral and/or motor function (AAN Task Force, 1991). Brain atrophy, both cortical and central, in AIDS patients has been detected in both CT and MR studies (Pedersen et al., 1991, Pan et

al., 1992). Analyses performed on autopsy material from AIDS patients indicate an 11% reduction of the neocortical volume and a 55% increase in the mean ventricular volume (Oster et al., 1993).

A stereological study of neocortex found a decrease in total neuron number of up to 30% (Oster et al., 1995). The study reported loss of neurons independent of whether or not the patients were clinically demented or signs of HIV encephalitis had been found in the brains. In light of the large loss of neurons in the neocortex, the aim of the present study was to investigate whether a neuron loss in the hippocampus could take place without causing symptoms of clinical dementia. Unbiased stereological methods for estimating the total number of neurons in the five subdivisions of the hippocampal region have previously been described in detail by West and Gundersen (1990), West et al. (1991), and Simic et al. (1997), and these methods were used to evaluate potential neuron loss in the hippocampus.

MATERIAL AND METHODS

Estimates were made on the total number of neurons in each of the five major subdivisions of the hippocampal region (dentatus, CA1, CA2/CA3, CA4, and subiculum) of nine male AIDS patients (age 20-59), who had displayed no symptoms of dementia, and 10 age-matched male controls (age 21-60) with no history of HIV infection, psychiatric disorders or neurological disease. One AIDS hippocampus was excluded from the material due to signs of Progressive Multifocal Leukoencephalopathy (PML), thus nine AIDS hippocampi have been included in the study.

The material from AIDS patiens was obtained from two municipal hospitals in Copenhagen in accordance with Danish laws governing the use of postmortem tissue in research. Patients with CNS opportunistic infections or neoplasms, drug addicts, alcoholics and clinically demented patients were excluded from the study. There were no neuropathological signs of acute ischemia in any of the brains (e.g. small red neurons in the CA1 region of the hippocampus).

The left hemisphere was embedded in agar and cut into 4.54-mm-thick slabs in the frontal plane. The region of the slabs containing the hippocampal formation was isolated and further divided into thinner slabs (2.27 mm) by an additional cut in the frontal plane resulting in 15 - 20 slabs from each patient. Each slab was embedded in glycolmethacrylate, and a 70-μm-thick section was cut from the corresponding face of each block. The sections were stained with a modified Giemsa stain and used for cell counting (Braendgaard et al., 1990).

Total neuron number

The total number of neurons was estimated in the following five subregions of the hippocampus: 1) the granule cell layer of the dentate gyrus, 2) the hilus of the dentate gyrus, 3) the pyramidal cell layer of CA3 and CA2, 4) the pyramidal cell layer of CA1, and 5) the pyramidal cell layer of subiculum. Delineation of the regions was performed on coded glass, using the same definitions as West and Gundersen (1990).

An unbiased estimate of the total number of neurons in each hippocampal subdivision was obtained with the optical fractionator method. This method combines counting, performed with optical disectors, with uniform systematic sampling (Gundersen, 1986; Gundersen et al., 1988) and has previously been applied to rat hippocampus (West and Gundersen, 1990; Korbo et al., 1996). The optical fractionator was chosen as an optimal method in a complex region as the hippocampus. The optical disector setting consists of a modified BH2 Olympus microscope connected to a video camera which

transmits the microscopic image to a monitor. A computer generated counting frame is superimposed on the screen using a CAST-GRID software system (Olympus, Denmark). A Heidenhain MT2 microcator is used to measure the movements in the z-direction. Two sets of motors are connected to the microscope to move the section at known distances in the x- and y-direction. The optical disectors constitute a known fraction of the volume of the region being analyzed. The area of the counting frame is a fraction of the area associated with each step in the x, y direction (the area sampling fraction, ASF). The height of the disector constitutes a known fraction of the thickness of the slab (the block sampling fraction BSF), since no shrinkage was found of either the thickness of the slab after plastic embedding or of the thickness of the section after histological handling (see below).

Each optical disector can be thought of as a series or stack of disectors (Sterio, 1984) generated at a particular position within the structure of interest. The series is produced by moving the focal plane of a high numerical aperture lens (100X oil, NA = 1.40) through a known distance of the thickness of a relatively thick histological section. Objects that come into focus within an unbiased counting frame (Gundersen, 1977) superimposed on the image at the focal plane are counted, Q^-. The total number of neurons (N) can be calculated as follows:

$$N = \Sigma Q^- \times 1/ASF \times 1/BSF \qquad (1)$$

Approximately 145 neuronal nuclei were counted in about 100 optical disectors in each subdivision using the sampling schemes as described by West and Gundersen (1990). The optical disectors with a height (h) of 20 μm were confined to the central portion of the section thickness (t) of 70 μm to avoid edge effects. The cells were identified as neurons if they had a nucleolus, a typical chromatin pattern in the nucleus, and were surrounded by cytoplasm.

The method is independent of tissue shrinkage in the x- and y-axis of the tissue. To ensure that there was no difference in shrinkage in the z-axis in any of the groups, one hippocampus from each group was selected for shrinkage estimation. Every third plastic embedded slab was sawed into four or five pieces and these were orientated perpendicularly to the cut surface. The pieces were reembedded in plastic, one section was cut, and the net shrinkage was calculated as the difference in slab thickness before and after plastic embedding. The shrinkage was 2-3% and considered to be negligible.

At the position of every 10th disector sample, the thickness of the section (i.e. the distance between the upper and lower surface of the section) was measured. The mean section thickness for each hippocampus was estimated from these measurements, and the mean section thickness was 1-2% below the expected (from the microtome), so the shrinkage was ignored.

Volume estimation

For the purpose of being able to compare macroscopic volumes, the volume of each subdivision of the hippocampus was estimated by point counting using the Cavalieri principle (West and Gundersen, 1990; Gundersen, 1986). After delineation, each section was looked up in a dissection microscope at 10X and a test system of points was placed randomly on each section. For hilus and CA3/2 a test system with an associated area per point (a(p)) of 1.0 mm^2 was used, while for CA1 and subiculum a(p) was 2.25 mm^2. Estimation of the volume of the granule cell layer was performed using a projection microscope with a magnification of 20X and a test system with a(p) = 0.22 mm^2.

Statistics

Student's ttest with a significance limit of $p< 0.05$ was used to evaluate differences in the mean of the number of neurons in each subdivision of the hippocampal region of the two groups. The appropriateness of the sampling scheme was evaluated by determining the degree to which the observed relative group variance was due to the precision of the individual estimates. The evaluation of the precision of the estimates, the coefficient of error (CE = SEM/mean), provides the information necessary for determining whether more or less sampling should be carried out at the various levels of the sampling scheme. The sampling is considered optimal, when the observed variance of the individual estimate, CE^2 is less than half the observed interindividual variance, CV^2, where CV = SD/mean. Calculation of CE in systematic sampled sections can be seen in Gundersen and Jensen (1987).

RESULTS

The total number of neurons and the volume of the five subregions are shown in Table 1. No difference in total neuron number or in volume of the subregions was found between the AIDS patients and the controls. As can be seen from the CV for the different subregions, there is a great interindividual variation in the total number of neurons in the human hippocampus.

Table 1. Mean total neuron number and mean volume of the different hippocampal subregions of non-demented AIDS patients and controls

	Total neuron number (10^6)		Volume (mm^3)	
	AIDS (n=9)	**Control** (n=10)	**AIDS** (n=9)	**Control** (n=10)
Granule	17.0 (0.26)	17.9 (0.26)	60.9 (0.17)	60.4 (0.20)
Hilus	1.75 (0.39)	2.12 (0.27)	201 (0.28)	243 (0.24)
CA3	2.99 (0.30)	2.71 (0.17)	168 (0.26)	157 (0.21)
CA1	13.3 (0.33)	14.7 (0.14)	808 (0.22)	821 (0.14)
Subiculum	5.11 (0.13)	5.85 (0.33)	439 (0.16)	467 (0.35)

Coefficient of variation (CV = SD/mean) shown in brackets.

The CE for the estimates was 0.08-0.15, giving a mean CE of 0.13 for both groups for the estimation of total neuron number in the subdivisions and a CE of 0.11 for the volume estimation for both groups. The ratio CE^2/CV^2 was 0.23 indicating that the sampling scheme was appropriate for this study.

DISCUSSION

With the present study we wanted to investigate a possible neuron loss in the hippocampus of HIV brains. Our results showed no significant difference in the hippocampal neuronal number in the HIV patients and control brains. The neuron number and the large interindividual variation in total neuron number found in this study is

concordant with previous stereological studies on the human hippocampal tissue (West and Gundersen, 1990; Simic et al., 1997). Our data are in agreement with some previous quantitative studies in AIDS brains where no change in neuronal density in the hippocampus neither in a subgroup with HIV encephalitis nor in a group with minimal neuropathological changes were reported (Spargo et al., 1993). Several studies have found a loss of neurons in neocortex from both demented and non-demented AIDS patients (Oster et al., 1995, Ketzler et al., 1990; Everall et al., 1991, 1993, 1994; Weis et al., 1993). The reduction in neuron number has been reported to be of the same size in the frontal, parietal, temporal and occipital neocortex (Oster et al., 1995). Despite the fact that those studies did not consider and analyse the neuronal number in the hippocampal formation, it is likely that hippocampal neurons are spared in the AIDS patients with no signs of dementia. Furthermore, the neuronal cell loss in the AIDS brain is a selective process which depends on the region and subpopulation of neurons affected (Lipton, 1996). The neuropathological process in the AIDS brain is located primarily in cortico-subcortical and white matter areas and correlated with appearance of dementia symptomatology (Asare, 1996). The results from this study address importance of hippocampal neuronal loss in relation to appearance of dementia in the AIDS brain. This hypothesis has to be tested using a similar methodological approach in the hippocampal region of demented AIDS patients. There are neuropathological conditions leading to dementia where the hippocampus is affected at an early stage such as Alzheimer's disease (West et al., 1994), Pick's disease (Ball, 1979), and Huntington's disease (Spargo et al., 1993). Particularly in the AD, appearance and non-random spreading of neuropathological changes from the hippocampus to the cortex correlates with the different grade of dementia symptomatology (Braak and Braak, 1991). Moreover, during the "normal" aging a selected neuronal loss in some of the hippocampal subfields was reported: hilus and subiculum or CA1 and subiculum (West, 1993; Simic et al., 1997), correlating with appearance of the senescent-related decline in memory function (e.g. benign senescent forgetfulness) (Hof and Morrison, 1994).

In conclusion, using the novel stereological method of the optical fractionator, we were able to determine the total number of neurons in each hippocampal subregion of the AIDS and control brains. The results obtained indicate that there is no hippocampal neuronal loss in non-demented AIDS brains. This might further strengthen the understanding of the dementia complex as a disease-specific pathological process in AIDS brains (Corder et al., 1998), differing in nature from morphological substrate of other degenerative dementia diseases.

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