ACTA STEREOL 1993; 12/2: 317-320
PROC 6ECS PRAGUE, 1993
ORIGINAL SCIENTIFIC PAPER

NO NEOCORTICAL NERVE CELL LOSS IN BRAINS FROM CHRONIC ALCOHOLICS

Grethe Badsberg Jensen and Bente Pakkenberg
Neurological Research Laboratory, Bartholin Instituttet,
Kommunehospitalet, Copenhagen
& Stereological Research Laboratory, Aarhus University,
Denmark

ABSTRACT

With the development of the new stereological techniques it has now become possible to make a precise and unbiased estimate of the total number of neurons in the complex human neocortex. In a study of 11 chronic alcoholic and 11 control male subjects there was no difference in nerve cell number in the neocortex between the two groups (Badsberg Jensen and Pakkenberg, in press). Macroscopic brain volumes were estimated and two statistically significant differences were found, namely an 11% reduction in the volume/weight ratio of white matter and a 30% reduction in the volume/weight ratio of archicortex in the alcoholics compared to controls. The volume of the ventricles in the alcoholic group was enlarged by 26%, which was not statistically significant. There was no difference in the volumes of the neocortices.

Key words: alcoholics, brain cortex, Cavalieri, neurons, optical disector

INTRODUCTION

During the last decade our knowledge respecting the effects of chronic alcohol abuse on the human central nervous system has increased considerably. Being lipophilic alcohol easily penetrates the blood-brain barrier, and its acute toxic effect on the CNS is well-known (Charness et al.,1989). Nevertheless, we do not fully understand the mechanism behind the toxic effects of chronic alcohol abuse. A common view is that alcohol abuse results in a diffuse loss of neocortical neurons, but very little is known about the pathology of cortical neurons (Thompson, 1982, Harper and Kril, 1990).

The aim of this study was to estimate the total number of neocortical neurons in chronic alcoholics using stereological methods.

MATERIAL

A group of 11 chronic alcoholics was matched with a group of 11 control subjects with respect to age (alcoholics: 46.8 yrs (range 36 to 64), controls: 46.9 yrs (range 34 to 67)) and body height (alcoholics: 178.8 cm (range 167 to 190), controls: 176.5 cm (range 162 to 186)).

Changes in tissue volume between the volume estimate and the numerical density estimate were quantified and found to be the same in the two groups: a swelling of 7.5% and 6.1%, respectively.
For further details see Badsberg Jensen and Pakkenberg, in press.

RESULTS

Brain weights were the same in the alcoholics and the controls (1443 gr vs. 1415 gr, p = 0.71) as were neocortical volumes (498 cm^3 vs. 486 cm^3, p = 0.66). The figure shows the two statistically significant differences that were found, namely an 11% reduction in the volume/weight ratio V_w of white matter to brain weight and a 30% reduction in the volume/weight ratio V_w of archicortex to brain weight in the alcoholic group compared with controls. No difference in the volume/weight ratio of neocortex to brain weight was found, see Fig. 1.

The volume of the ventricles in the alcoholics was enlarged by 26%, a difference that did not reach statistical significance.

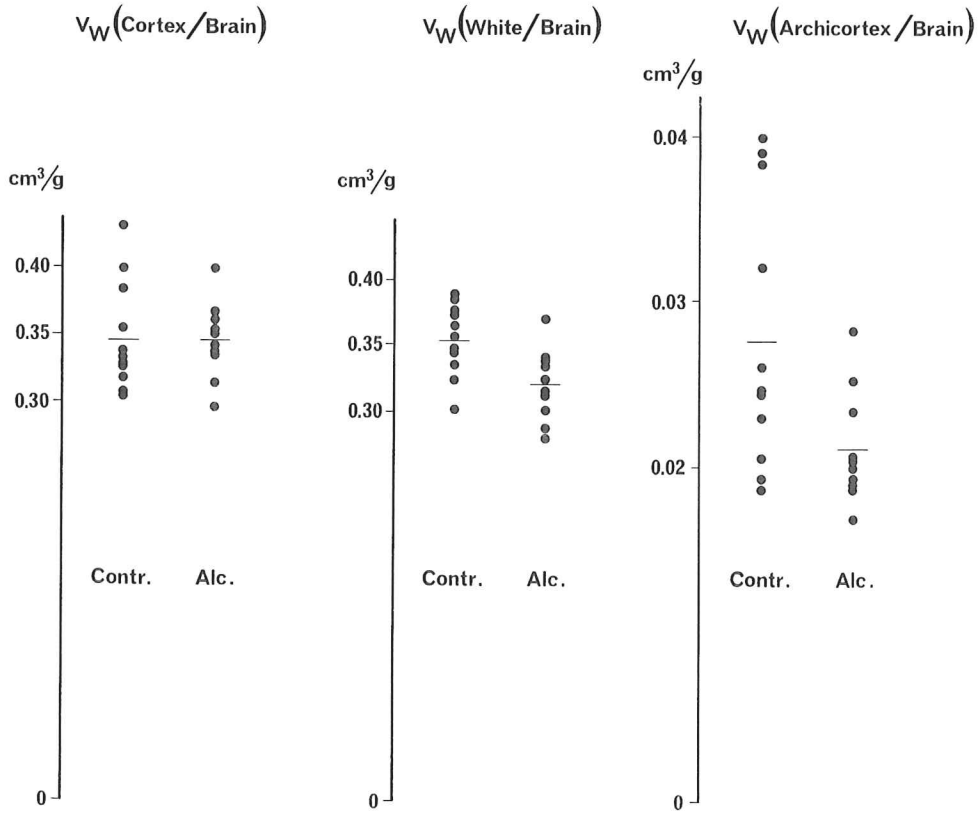

Fig. 1. Volume/weight ratio, V_w of neocortex, white matter, and archicortex.

All alcoholics had pathoanatomical evidence of alcohol abuse and most had liver cirrhosis and varices of the oesophagus. Excluded were patients with hepatic coma for more than five days, females and individuals older than 67 years. Other exclusion criteria were signs of infarcation, neurological diseases other than those associated with alcoholism, patients taking CNS-medication, diabetics, patients with disseminated cancer, severe lung diseases, a history of severe head injuries, withdrawal seizures or episodes of delirium tremens leaving a total of 11 chronic alcoholics for further examination.

Control subject were selected according to the above-mentioned criteria, except that they did not have any history or histological signs of alcohol abuse.

METHOD

All brains were fixed in 0.1M sodium phosphate buffered formaldehyde. Right or left hemispheres were chosen systematically randomly. The brain volumes were estimated by the Cavalieri method and counting performed using a uniform sampling design and optical disectors (Brændgaard et al., 1990).

The hemispheres were embedded in 6% agar, sliced coronally at 7 mm intervals and the neocortical volume was estimated by pointcounting. From every second neocortical slice, starting randomly, transcortical wedges were sampled uniformly from each neocortical region. Each wedge was cut into 2-mm-wide parallel bars providing 25 to 50 bars per region. These were subsampled uniformly so that each region was represented by 6 to 10 bars. Each bar was embedded in LKB Historesin® from which one 35 μm thick section was cut and stained with modified Wolbach's Giemsa and used for counting in an optical disector.

The disector is a probe which samples isolated particles with a uniform probability in a three-dimensional space, irrespective of their size, shape or orientation. The disector needs pairs of thin sections for numerical estimation. In the optical disector thick sections are used, e.g. 35 μm, and the plane of focus is moved up or down inside the section. To estimate the number of particles one counts the particles in the disector, Q^-. Knowing the height h of the disector and the area of a counting frame $a(frame)$, the volume of the disector, $v(dis)$, is given as:

$$v(dis) = h \times a(frame) \qquad (1)$$

The total number of particles $N(part)$ in a specimen of a given volume $V(ref)$ is:

$$N(part) = \Sigma Q^- / \Sigma v(dis) \times V(ref) \qquad (2)$$

The optical disector counting equipment consists of a BH-2 Olympus microscope with a motorized stage movement, and an electronic microcator with digital readout for measuring movements in the Z direction. High image resolution and a thin focal plane are obtained using a high numerical aperture (NA 1.4) and a 100x oil immersion objective. Using the GRID program (BICO, Denmark) and an AMIGA 2000 computer, counting frames (average area 384 cm^2) were superimposed by video camera/Genlock connection on to a colour monitor where the actual counting took place at a final magnification of 3900x.

Table 1. The table shows total neuron number (10^9) in neocortex and the four different neocortical regions.

	Controls N = 11	Alcoholics N = 11	p
Frontal	8.22 (0.18)	8.05 (0.23)	0.82
Temporal	5.34 (0.075)	4.93 (0.23)	0.27
Parietal	5.25 (0.22)	5.64 (0.095)	0.33
Occipital	4.42 (0.19)	4.83 (0.18)	0.28
Total Neocortex	23.2 (0.12)	23.4 (0.12)	0.86

Mean values and Coefficient of Variation (CV = SD/mean) are given.

DISCUSSION

The total number of neurons has been estimated in neocortices from chronic alcoholic patients; no global nerve cell loss was found.

Our study indicates that chronic alcoholics lose white matter, and this could provide the basis for their functional impairment. Yet the results suggest that the observed brain damage in the alcoholics is potential reversible since preserved nerve cell bodies might, at least in principle, allow lost or malfunctioning neurites to be reestablished and restore function after prolonged abstinence and/or treatment. In contrast, lost neurons in neocortex cannot be replaced.

Still cell losses in other brain areas, such as hippocampus and subcortical limbic areas cannot be excluded, which future studies will have to determine.

REFERENCES

Badsberg Jensen G, Pakkenberg B. Do alcoholics drink their neurons away? The Lancet, in press.

Brændgaard H, Evans SM, Howard CV, Gundersen HJG. The total number of neurons in human neocortex unbiasedly estimated using optical disectors. J Microsc 1990;157:285-304.

Charness ME, Simon RP, Greenberg DA. Ethanol and the nervous system. New Eng J Med 1989;321:442-54.

Thomson AD. Alcohol-related structural brain changes. British Med Bul 1982;38:87-91.

Harper CG, Kril JJ. Neuropathology of alcoholism. Alcohol & Alcoholism 1990;25:207-16.